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PHLOEM ANATOMY, EXUDATION, AND TRANSPORT OF ORGANIC NUTRIENTS IN CUCURBITS

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(WITH SIX PLATES AND ONE FIGURE)

Introduction

A survey of the subject of translocation of organic nutrients, in recent botany texts, indicates that mass flow through the sieve tubes, facilitated by perforation of the sieve plates, is the prevailing concept. Several authors distinguish between carbohydrates and proteinaceous materials as to their channels of movement, and some avoid the subject altogether. A majority favor this time-honored concept of the mechanism, however, although it has been seriously questioned in recent years.

Since the early descriptions of sieve tubes by HARTIG (**18**) and NÄGELI (**31**), the cucurbits have been widely used in the study of phloem anatomy. The clear preparations which may be obtained from these plants have undoubtedly been instrumental in the development of the sieve-tube theory of translocation. The ease of demonstrating phloem exudation in these plants is another factor which has strengthened the concept of mass flow through sieve tubes (**30**).

HARTIG described the sieve tube in 1837, gave an account of the sieve plate in *Cucurbita pepo* in 1854 (**18**), and described experiments with phloem exudates in 1858 (**19**). He considered the protoplasmic connections in the sieve plate to be uninterrupted, but VON MOHL (**29**) opposed this idea, holding that the pit-closing membrane is intact.

NÄGELI (**31**) gave an accurate description of the sieve plate; he undoubtedly observed callus formations; and he attributed a physiological significance to the characteristic slime accumulations so often observed in these tissues. He considered the sieve plate to be actually perforated, and thought that the slime accumulation was caused by a filtering of large particles from the moving sap. Under excess pressure the slime might even be forced through the pores according to his description. He proved that

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the exudate from these stems comes from the phloem, and used this as additional evidence for a mass flow through the sieve tubes.

SACHS (**34**) advocated the idea that sieve tubes conduct only proteinaceous materials, carbohydrates moving through the remaining phloem elements.

The mechanism proposed over sixty years ago by these early workers is the one which is described in botanical text-books today. Subsequent ringing experiments have strengthened this view, while the occasional objections which have been proposed have been either overlooked or neglected.

The early works on sieve tube anatomy were collected by DE BARY (**2**), and later studies are well summarized by LECOMTE (**25**), PERROT (**32**), and HILL (**20**). It suffices to say here that they left unaltered the concept of mass flow in the sieve tubes. FISCHER (**14**) showed that the slime accumulations noted by NÄGELI were due to manipulation of the material, but it remained for LECOMTE (**25**) to give a proper interpretation of their formation. The latter work has been confined and extended and will be described in a later section. STRASBURGER (**41**) considered the sieve pores of angiosperms to be open, while those of certain gymnosperms are closed by median knots which he compared in permeability to the pit-closing membranes of bordered pits of xylem vessels.

CZAPEK (**8**) thought that all organic materials pass through the sieve tubes, but HABERLANDT (**16**) states, as did SACHS (**34**), that only nitrogenous compounds move in these elements. Physiologists still seem to favor the view that organic nutrients are conducted through the sieve tubes by a mass flow (**30, 36**) or by some sort of streaming movement (**6, 27**).

Anatomists (**2, 25, 40, 20**), while agreeing consistently that the protoplasm of the sieve tube is continuous through the sieve plate, have failed to question the existence of intervacuolar pores within the protoplasmic strands. They have considered that translocation and phloem exudation prove their existence. This attitude is well expressed by HILL (**20**), where on page 285 of his summary he says: "In the sieve-tubes there is the need of the formation of definite holes for the translocation of material, and the connecting threads serve as useful paths along which to work in order to produce tubes of sufficient diameter to enable an actual flow of slime to take place. Then, since the large holes produced are likely to be a source of danger to the plant when translocation diminishes the callus is utilized to regulate the bore of the tubes, and finally appears to put a stop to translocatory processes altogether." On page 262 of the same publication the sieve plates of *Vitis* are diagrammed, a clear distinction between slime and protoplasm being shown. In this author's own drawings such clear distinction is not shown, and in reading the text, the basis of differentiation is found to lie in the staining reactions of the connecting strands. In HILL's

words (page 267), "The contents of these fine tubes are found to react in a different manner to stains after the appearance of the callus, for the protoplasmic threads of the embryonic condition have now become the more important slime strings of the mature sieve tube....."

More recent works on anatomy (**40, 11**) express a similar view on the structure of the protoplasmic connections of the sieve plate; in fact, the idea seems prevalent that these tubelike connections occur in most plants and that they provide adequate channels for longitudinal conduction.

There are two possible objections to this view, based on features of the sieve structure. If the middle lamella of the sieve plate actually closes the simple pits through which the protoplasmic connections pass (**29, 41, 25, 31**), mass flow would be seriously hindered. PERROT (**32**, p. 116) considered this of only secondary importance, and since the cellulose mesh of the plates of obliterated sieve tubes is actually perforated, it seems that at least during the more mature stages actual continuity through the pores is established. SCHMIDT'S (**35**) work seems to confirm this, although the effect of the reagents used upon such a thin structure is somewhat doubtful.

The question as to the existence of intervacuolar connections or pores through the protoplasmic strands of the sieve plate is more serious. KUHLA (**24**) thought the connections to be solid and SCHMIDT'S work indicates the same. The writer agrees with SCHMIDT on this point. In the first place, the actual protoplasmic strand is very narrow, approaching in most cases the limit of the resolving power of the microscope, so that differentiation is extremely difficult. In the second place, it is doubtful whether the reagents used will satisfactorily distinguish between protoplasm and vacuolar content in such minute strands.

Physiologists have apparently accepted the anatomists' view of sieve-tube structure until recently. RUHLAND'S (**33**) studies on the permeability of sieve tubes aroused little comment, but the works of SCHMIDT (**35**), BIRCH-HIRSCHFELD (**4**), DIXON and BALL (**9**), DIXON (**10**), and CRAFTS (**5**) indicate from varying angles an increasing doubt as to the function of sieve tubes in translocation. The last paper, while giving little definite proof, strongly indicates that the sieve-tube mechanism is inadequate to explain longitudinal conduction in certain plants. The present paper takes up a more detailed study of sieve-tube anatomy in relation to exudation and translocation.

Phloem anatomy in cucurbits

It is evident that if a clear idea is to be obtained of the relationship of the different phloem elements to translocation, detailed studies of phloem anatomy are needed. This type of study precludes the use of many differ-

ent species, and in the following work only two, *Cucurbita pepo* and *Cucumis sativus*, have been used.

In studying the phloem of cucurbits, it soon becomes evident that there are two distinct types of sieve tubes which, although having many features in common, can readily be differentiated on the basis of their structure and contents as well as on their location within the stem. In the transverse section of the stem of *Cucurbita pepo* diagrammed in text figure 1, the bicollateral vascular bundles and various sieve tubes are shown. FISCHER (**12, 13,**

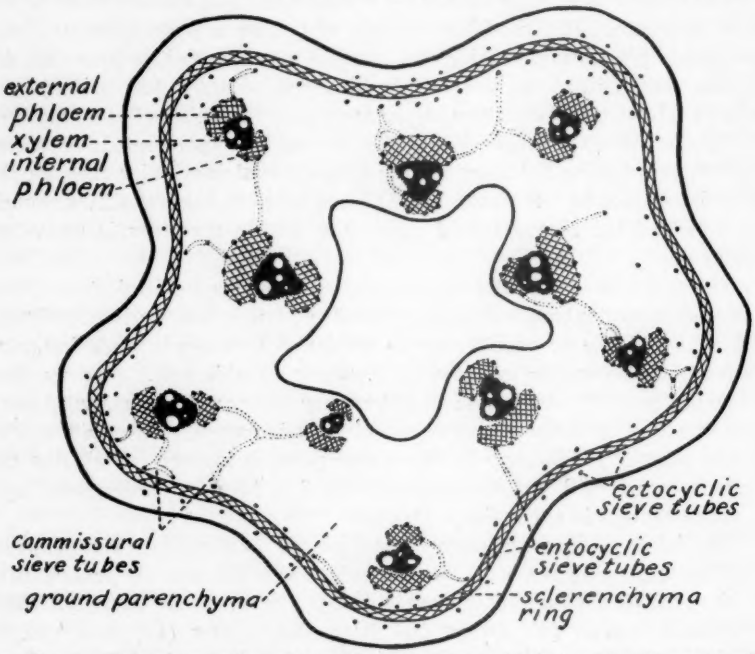

FIG. 1. Transverse section of pumpkin stem showing bicollateral bundles, ectocyclic and entocyclic sieve tubes. Commissural tubes follow curved and diagonal paths through the ground parenchyma. These paths as determined from serial sections are dotted in. Ectocyclic and entocyclic tubes are united only at nodes of stem.

15) has made a careful study of the sieve tubes of cucurbits and his nomenclature is used in this figure. In addition to the tubes of the vascular bundle, he distinguishes "ectocyclic tubes" lying between the epidermis and the sclerenchyma ring, "entocyclic tubes" lying inside this ring, and "commissural tubes," connecting the latter with those of the phloem groups of the bundle and connecting the peripheral tubes of the various bundle groups.

These various tubes are shown in more detail in figures 1 and 2, and a distinction may clearly be made. The inner or central tubes of the phloem groups (fig. 1) are practically devoid of stainable contents, while the peripheral tubes of the groups (fig. 1) and the commissural and entocyclic tubes are densely filled.

On the basis of structure alone these various elements would fall into three groups: (1) the central tubes of the phloem groups of the vascular bundles, having short broad elements comparatively poor in stainable contents; (2) the longitudinal tubes of the periphery of the phloem groups, the ectocyclic tubes and the entocyclic tubes, all having on an average narrower and much longer elements and, in the mature state, densely staining contents; (3) the commissural tubes, varying greatly in length but resembling the second group in diameter, content, and sieve-plate structure, and connecting laterally the various longitudinally arranged tubes of the stem. Since the latter two groups are similar except in average length of elements and in position in the stem, two features which hardly allow for a physiological differentiation, they will be classed as one type in the following discourse, although the names given by FISCHER may occasionally be used.

The tubes lying in the center of the phloem groups will be mentioned first since they have been most widely described in the literature. The first distinguishing characteristics of the young differentiating sieve tubes in this material are the slime drops and the pitted end walls. The slime drops have been described and illustrated by FISCHER (**12,** fig. 9, Pl. I; fig. 4, Pl. II; fig. 9, Pl. IV; **15,** figures in Pls. I and II), LECOMTE (**25,** figs. 26 and 27, Pl. XXII), and others. They are translucent plastid-like bodies which lie in the parietal protoplasm of the developing cell. They can be distinguished during the early stages of vacuolation, before the cell has attained full size and while the nucleus is still prominent. At this time the protoplasm shows active streaming and the vacuoles will accumulate neutral red, but no protoplasmic connections can be distinguished traversing the end walls. It is interesting to note, however, that the middle lamella tends to stain deeply with neutral red, and by focusing carefully it can be seen that the portions occupied by sieve fields do not stain while the intervening portions do. This indicates that the sieve field regions differ in composition at an early stage.

In stems killed in hot water or 50 per cent. alcohol, the slime drops are fixed and stain readily with anilin blue (figs. 3, 4, 5). As the elements reach mature size, these structures enlarge and small vacuole-like spots become evident within them (figs. 6, 7, 8). At the same time the protoplasmic strands of the sieve field can be distinguished, and the first traces of the callus cylinders which eventually surround the strands appear (fig.

26). The nucleus is still present, but is larger and less deeply staining; the vacuoles unite to form a large central vacuole and protoplasmic streaming and neutral red accumulation diminish. As the elements reach maturity several rather complicated changes take place rapidly, making accurate description difficult. The sieve plate thickens, the original protoplasmic connections apparently fuse to form a single strand in each field, and the callus cylinders enlarge and elongate as described by HILL (**20**).

In the meantime that portion of the parietal protoplasm lying against the wall apparently increases in viscosity and ceases to stream, while anastomosing strands on the inner surface still show sluggish plastic movements. The slime drops enlarge further, the vacuoles within them increase in size and number, and stain is absorbed to a lesser degree in fixed material. The tendency for neutral red to accumulate in the vacuoles becomes much less pronounced at this time, although an accumulation by the vacuoles in the slime drops indicates that it is present in the protoplasm.

As the protoplasm becomes set on its outer surface, the inner strands tend more and more to push out from one slime drop to another across the vacuole (figs. 6, 7) and form an anastomosing network similar to that of the cells of epidermal hairs. An attempt has been made to illustrate this in serial order (figs. 8–12), but it is difficult since in fixed material the strands of the protoplasmic network within the vacuole shrink to mere threads and are not easily stained. This inner network of protoplasmic strands becomes fixed to the sieve plates of the end and side walls, loses its fluid consistency, and becomes relatively inert.

The existence of this inner structure in sieve tubes has been the subject of some controversy. FISCHER (**13**) disagreed with WILHELM'S assumption of an inner sheath limiting the slime accumulation, or slime plug, considering it to be simply a slime formation. He later (**15**) described the contents of the sieve tube of cucurbits, making no mention of such an inner structure. ZIMMERMAN (**43**) seemed to find evidences of an inner structure which stained differently from the plasma sheath. He found it present in several species and in sieve tubes in different stages of maturity. The confusion which exists in relation to these inner threadlike structures can readily be understood, since any killing agent which causes a strong contraction of protoplasm will rupture them and collapse the outer sheath around the inclosed slime, completely obscuring them (fig. 19). An agent which kills very slowly, on the other hand, might cause a slow cessation of the plastic flow by which they are formed and so eliminate them before the cell is killed. In young living sieve tubes they are broad (fig. 6), tending to become more threadlike and numerous with maturity (figs. 7–14, 17, 18, 27, 28, 32, 33). They are seen only with difficulty in such tissues, and great care is required in preparation of sections as injury stops the flow of proto-

plasm in many cases. On the other hand, certain agents stimulate protoplasmic streaming (**37**), and this may explain the results obtained with low concentrations of alcohol as a killing agent in rendering these structures visible in fixed material.

The nucleus loses its staining properties as the elements mature, and apparently disintegrates, and neutral red is no longer accumulated within the vacuole. The slime drops, while tending to adhere to the protoplasmic strands of the vacuole, gradually swell, vacuolate, and disintegrate (**15,** p. 302), so that they become fewer in number and finally disappear as discrete bodies. The material resulting from disintegration of the nucleus and slime drops forms a colloidal suspension in the vacuole, and it is the coagulation and aggregation of this material which makes up the amorphous content of slime plugs in mature sieve tubes (figs. 27, 30).

The sieve plates assume their mature form, each protoplasmic strand being surrounded by a callus cylinder which projects beyond the level of the cellulose base. These cylinders may be seen (fig. 23) in transverse sections in which the knife has passed close enough to the plate to clear it of adhering protoplasm. By slightly raising the focus, the protoplasmic strand disappears and a light spot is seen in its place (fig. 24), due to an optical effect. The fact that DE BARY (**2**) mentions 5 μ for the maximum diameter for the sieve pores, while the largest protoplasmic connections measured in this work never exceeded 2 μ, indicates that the early workers possibly thought the callus cylinder to be the protoplasmic connection and the actual plasma strand to be a pore. This possibility is further indicated by the labeling of figure 65 *n* and 66 *l* on page 174 of the English translation of his book (**2**). Here he calls the structure comparable to the dark protoplasmic core in figure 23 an open pore. The sieve plate in figure 23 is shown in figure 25 with side illumination, bringing out even more the crater-like appearance of the ends of the callus cylinders which surround the protoplasmic connections.

In the development of definitive callus, these cylinders increase in diameter as they lengthen until they fuse laterally, forming a perforated plate (**15**) which increases in thickness, stretching the protoplasmic strands to many times their original length (fig. 31).

In all of the preparations which have been made in this work, there has not appeared a single indication of the presence of two phases within the protoplasmic connection of the sieve plate. The protoplasm changes in its staining reaction just as it does in its other physical properties. No evidence has been brought to light, however, which justifies the assumption of a "protoplasmic tubule" (HILL p. 261) and an inclosed "slime string."

Typical slime plugs occur only in the sieve tubes in the centers of the phloem groups. NÄGELI (**31**) observed these structures and proposed that

they were the result of sap movement. FISCHER (**14**) found that by killing intact plants in hot water he could avoid their formation. He concluded that they were due to the rapid sap flow resulting from cutting the stem and thus steepening immensely the pressure gradient in the tissue. LECOMTE (**25**), however, found that slime plugs did not occur in fresh material but were the result of treatment with alcohol. This work has been repeated and confirmed, and the same results have been obtained by placing cut stems in hot water or by touching the end of a fresh section on a slide with a hot needle. These experiments indicate that slime-plug formation results from the action of agents which kill protoplasm and render it permeable. Following this action, turgor pressure or phloem exudation forces a part of the contents of the sieve-tube element through the permeable portion of the protoplast, filtering out any coagulated material.

Slime-plug formation is shown in sieve tubes in different stages of maturity in the series figures 11–16, the last being the appearance after the slime drops have almost completely disintegrated. In figure 29 the parietal protoplasm is shown in transverse section pulled slightly away from the walls. The slime plug shown in figure 28 is distinct and separate from this parietal protoplasmic layer, and at the lower end of the plug two strings of the inner meshwork may be seen. In figure 27 protoplasmic strings are shown traversing the total length of the cell. Three distinct strings can be seen emerging from the slime plug on the lower left side, while the parietal protoplasm remains but slightly removed from the walls. In figures 17 and 18 are shown two sieve tubes with slime plugs formed by killing with 50 per cent. alcohol. The parietal protoplasm remains practically intact and strings may be seen attached to the pits in the side walls. Killing with 95 per cent. alcohol results in more drastic shrinking, and the failure of past workers to distinguish between the parietal protoplasm and the internal meshwork is probably due to its use. In figure 19 the result of this protoplasmic shrinkage is shown, while in figure 20 the protoplasm has been torn completely free from the upper side of the plate, leaving small droplets where the strings have been broken.

By controlling the conditions, slime plugs may be formed on the two ends of a section pointing in opposite directions. When this is done, as by touching each end of a fresh section on the slide with a hot needle, the sieve tubes in the center of the section may show slime plugs in various positions (figs. 30, 32, 33, 36).

In the many stems used in this work, only one case was found in which slime accumulations could be seen in fresh material. This occurred in mature sieve tubes of a cucumber plant which was growing vertically on a string, and suggests that under certain conditions, after disintegration of the slime drops, suspended materials may gravitate to the lower ends of the

cells as do starch grains in the sieve tubes of many plants. It may be said with certainty, however, that formation and orientation of the slime plugs have no correlation with the previous direction of sap flow in the plant.

The small mucous droplets or bubbles on sieve plates, mentioned by LECOMTE (**25**) as indicating the movement of sap through sieve tubes, are apparently as poor a criterion as are the slime plugs. NÄGELI (**31**) pictures these structures very clearly and they were often observed in the tissues being studied by the writer. They were never found very far from the cut end of the sectioned stem, however, and appear to be strictly localized in places where the pressure gradient has been immensely steepened by cutting. They also are found only in sieve tubes in certain stages of development, and appear to result from the semifluid protoplasm of the protoplasmic connection being forced through the pore and being inflated with sap within the vacuole of the succeeding sieve-tube element. The suggestion that it indicates transport of sap is difficult to understand, since its very structure shows that it would hinder rather than allow mass flow.

In a recent publication (**22**) JUNGERS proposed that the structures referred to in the literature as protoplasmic connections are in reality wall structures. Several of the preparations used in this work seem to refute this idea, although positive proof is difficult to produce. By carefully focusing on the structures pictured in figure 31, they can be followed without interruption from the protoplasm of one cell to that of the other. In figure 21 is shown a mature sieve tube in a stem killed by the use of hot water. The protoplasm contracted slightly, drawing out the protoplasmic connections without rupturing them, and they may readily be followed from the surface of the protoplasmic mass to the pore in the sieve plate. LECOMTE shows a similar preparation (**25,** Pl. XXI, fig. 8). In staining a section of epidermis from the vein on the back of a cucumber leaf to show protoplasmic connections, the protoplast was plasmolyzed, and in pulling the protoplasm away from the wall threadlike strands were left joining the connections in the pits of the wall with the external surface of the protoplast (fig. 22). These preparations seem to indicate the protoplasmic nature of the strands traversing the pit-closing membranes. If they are not protoplasm they are at least intimately connected with the protoplast, so that it should be very difficult on the basis of a microchemical test to prove that they have no protoplasmic content.

The second type of sieve tubes of the cucurbit stem has several distinctive features. While the elements of the central tubes of the phloem groups vary between wide limits in diameter and tend toward a uniformity in length, those of this type range from 0.05 to 1 mm. in length and approach a mean diameter of 0.02 mm.

In the early stages of development the two types are very similar in the nature of their contents (figs. 3, 34), although the slime drops tend to be smaller in the second type. As they approach maturity, however, distinct differences may be noted (fig. 35). The internal protoplasmic meshwork (figs. 27, 28, 32) is lacking, although a rather fibrous tuft may be seen on the sieve plate (fig. 35). The sieve plate fails to thicken as noticeably as in the internal tubes, and the vacuolar contents take on a granular appearance and become more and more dense (fig. 36). As the dense granular mass increases to fill the vacuole, a metamorphosis starts from the parietal protoplasmic layer and gradually approaches the center, the granular contents changing to an amorphous layer which eventually fills the tube completely. In figure 38 this change can be seen starting at the sieve plate, and in figure 39 a layer encircles the granular contents in the center tube while in the one on the right the contents have become completely amorphous. The sieve plate does not thicken during this development, but when the contents have reached the amorphous state, definitive callus is laid down (fig. 37). As the tube ages the jell-like content becomes broken by cracks and fissures (fig. 37) (**13**, Pl. IV, fig. 8; **15**, Pl. I, fig. 34).

Companion cells are very prominent with these sieve tubes (figs. 35, 39) and always accompany them. The commissural tubes (figs. 2, 40, 41) are much shorter in length than are those which traverse the stem longitudinally, but in other characteristics they resemble them closely. They are apparently cut from dividing ground parenchyma cells, and serve to link up the ectocyclic and entocyclic tubes with the peripheral tubes of the phloem groups as well as to connect the different phloem groups of the stem.

Slime plugs have not been found in this second type of sieve tube, although a density gradient in the granular contents in fixed material (**13**, Pl. I, fig. 6; **15**, Pl. II, fig. 44) indicates that a similar action occurs following treatment with killing agents.

Phloem exudation in cucurbit stems

HARTIG (**19**) described studies on phloem exudation in 1858. The exudate from cucurbit stems and fruits was analyzed by ZACHARIAS (**42**) and KRAUS (**23**), and was found to contain from 7 to 11 per cent. dry weight, of which a large portion was organic nutrient material. NÄGELI (**31**) thought that this phloem exudation from cut cucurbit stems proved the occurrence of a mass flow in sieve tubes. FISCHER (**14**), LECOMTE (**25**), HABERLANDT (**16**), MÜNCH (**30**), and many others have used the same reasoning in support of the sieve-tube theory of translocation.

In the course of the present work many measurements have been made on the rate of exudation from phloem tissues. In a previous paper (**5**)

data were presented indicating an average rate of linear displacement of the total phloem volume of 0.8 cm. per minute for twenty collections, the maximum rate recorded being 3.5 cm. per minute. From another series of collections it was found that by successively cutting thin sections from the exuding end of a stem, exudation could be maintained for a considerable period of time. Several of the early collections, especially the one resulting in the high rate mentioned above, gave the impression that exudation was exceedingly rapid from young actively growing shoots. In order to carry this investigation further, a number of cucumber seedlings were grown in small pots and the young tender growing tips were dissected under a Greenough binocular. It was found that while exudation from the cut hypocotyl or elongated internode was comparable with that mentioned above, when the young tender tissues of the tip were severed an exceedingly high rate of flow resulted. The absolute amounts of exudate were not great, but in relation to the diameters of the structures cut, the spheres of exuding sap were large and developed with amazing rapidity. Tips of bracts, inclosing the young flowers, which were 0.5 mm. or less in diameter, would give rise to spheres of sap several times this diameter within a few seconds.

When a young leaf was cut the sap welled out along the entire cut surface, but when torn it could be seen in growing droplets upon the ends of broken veins. After removing several young leaves and flowers and allowing the escape of a relatively large amount of sap, new incisions would be followed by an apparently undiminished flow, as if the sap were coming from a large, elastic reservoir.

Histological examination of these young structures failed to reveal the presence of mature sieve tubes. In the younger bracts and leaves the phloem consisted of elongated parenchyma cells such as are described in bundle terminals by FISCHER (**13**) and STRASBURGER (**41**). The sieve tubes of the young stem were nearly all in the slime drop stage, and in fresh sections they accumulated neutral red. When plasmolyzed by killing in strong IKI solution, the protoplasm pulled away from the end walls as might be expected in ordinary elongated parenchyma cells.

Exudation from mature tissues proved to be most rapid from the peduncles of developing fruits of the cucumber, and rates will be given in the following section. Extending the observations on the period during which exudation could be maintained, several cucumbers weighing 200 gm. or more were cut from the plants, and by repeated cutting of the peduncles at intervals of about 15 minutes, continued exudation was induced. One fruit was still exuding after four hours of such treatment and another continued for two and one-half hours. When the fruits were inverted with their cut peduncles immersed in water, strings of coagulated exudate 6

inches or more in length could be seen hanging from the cut surfaces. When 50 per cent. alcohol was used, the coagulated exudate could readily be observed streaming down from the cut phloem groups.

Several full-sized cucumbers, having peduncles more than 1 cm. in length, were allowed to remain on the laboratory shelf for a period of six weeks, no precautions being taken to keep them cool or to prevent water loss. They were in diffuse light and the temperature averaged about 20° C. At the end of this period one of the fruits was examined for exudation by cutting off a piece of the peduncle about 3 mm. in length. The rate of exudation seemed normal, if not higher, so a collection was made on a second fruit, and the rate, given in detail in the next section, was found to be rather high. Upon sectioning the peduncles through which this exudation had occurred, it was found that all of the central sieve tubes of the phloem groups had large masses of definitive callus on their sieve plates, while the plates of the peripheral, commissural, and entocyclic tubes were free of callus with the exception of a few obliterated tubes. Tubes of the first type were practically free of stainable contents, while those of the second type were densely filled with a granular material, both in fresh and fixed sections. Masses of coagulated exudate adhering to the cut ends of the sections absorbed stain readily and had a colloidal structure which was practically amorphous, while the granules in the sieve tubes were similar in size to those shown in figures 34, 35, 38, and 39.

Green cucumbers hanging on plants which had matured and dried in the greenhouse were examined in a similar way, and, while exudation was still rather profuse the phloem proved to be in the condition just described. The central sieve tubes of the fruits, as well as those in the peduncles, proved to be callused.

In SCHUMACHER'S (**36**) recent work on translocation, he found the callusing of sieve plates to be a very sensitive reaction to immersion of leaves in eosin solutions. Finding the loss of nitrogen and dry weight from darkened leaves which had been so treated to be materially reduced, he concluded that the sieve tubes are the channels of transport for organic materials.

The effect of immersing cucumber leaves in eosin and in water, both in the light and dark, has been studied and the response described by SCHUMACHER has been confirmed. Practically all of the sieve plates in the main veins and petioles of the eosin-treated leaves were heavily callused within 48 hours, while immersion in water had no effect within that time. Leaves treated in the light reacted in the same way as those which were darkened, and cutting the veins was not essential to the response. The sieve plates of young sieve tubes in the slime-drop stage were callused as well as those of greater maturity. These young elements failed to accumulate neutral red

when their plates were callused, and no protoplasmic streaming could be seen in them. Callusing occurred in sieve tubes of both of the types described in the previous section, and the accumulation of granules at one end of the cell in the cortical tubes was not hindered by its presence. Slime accumulations in the central tubes of the phloem groups were smaller or entirely lacking. After an exposure of 96 hours to the eosin solution, exudation was stopped, and microscopic examination showed that not only was callusing complete but the protoplasm and vacuolar contents were contracted and the walls collapsed, indicating that the cells had been killed.

Callus formation is typically a response to injury or degeneration of the sieve-tube elements. SCHUMACHER found it near the cut ends of stems, and in the present work it has occurred many times in stems collected in the greenhouse and placed in tap water in the laboratory. It generally occurs in old sieve tubes during their decline and is typical of the phloem of stored fruits. LECOMTE (**25**) described experiments in which he found that cucurbit seedlings grown in the dark had definitive callus upon the sieve plates of all mature sieve tubes.

SCHUMACHER comes to the conclusion that eosin is not carried back through the xylem, but that extension of the response for as far as 30 cm. from the point of application may result from movement of the eosin, or a stimulus resulting from eosin treatment, through the sieve tubes. Eosin is a very toxic substance, being even more harmful to plant tissue than arsenic. The fact that the mesophyll cells remain uninjured for several weeks after treatment suggests that the eosin did not enter them, yet it could enter the phloem only through them if it did not go through the xylem.

Dissection studies indicate an exudation pressure in the phloem and a subatmospheric pressure in the xylem. Since the solution covering the leaves in these treatments disappears more rapidly than can be accounted for by evaporation, it seems that it must enter the xylem and slowly displace the normal xylem sap, moving back down the petiole and into the stem. Since transpiration continues in the petiole, and uptake by the phloem is not entirely hindered, eosin would be carried into all of the surrounding tissues. In transverse sections of the petioles of leaves which had been immersed in a 1:25,000 eosin solution for 96 hours, the stain could be seen in the walls of xylem, phloem, cortex, and epidermis. Tests to be described later have given an indication that rather early in their development sieve tubes exhibit an increasing permeability with age as compared with companion cells and phloem parenchyma. This offers a possible explanation for their being so much more severely affected by eosin solutions.

Failure to obtain the typical response in abscissed leaves allowed to absorb eosin solution by transpiration might be due to rapid killing, resulting from accumulation of the stain in the tissues. When the treatment is applied by

immersing abscissed leaves in the eosin solution, tension in the xylem is reduced as well as translocation in the phloem, so that injection of the stain could not be expected.

In the light of the experiments on exudation which have been described, it seems difficult to agree with the theory that definitive callus serves to control conduction by constricting the protoplasmic connections of the sieve plate (**16, 20, 25,** etc.), or that it may be of use to the plant in preventing the penetration of toxic substances (**36**). Since exudation is little affected by its presence or absence, the possibility of a relationship with perforation of the sieve plates seems even more remote.

The anastomosing system of sieve tubes which is found outside of the phloem groups of the vascular bundles resembles in several ways the open latex systems of many other plants. That it offered a ready explanation for the rapid flow of exudate seemed obvious, until a thorough study had shown that the end walls are typical sieve plates and that the parietal protoplasm persists throughout the life of these elements. The pores of the plates are very fine and are filled by protoplasmic strands. Thickening of the plate by callus is not so marked, and definitive callus is not formed until the contents have solidified, except from eosin treatment.

FISCHER (**13**) studied the development of this cortical sieve-tube system. stating that the elements were formed early in the development of the stem; that they functioned in the nutrition of the elongating tissues; and that obliteration occurred by the time the stem was fully mature. The writer's observations differ considerably from those of FISCHER. These tubes could be found fully developed in stems in various conditions from very young elongating internodes to peduncles of stored fruits. A few of them were found in the obliterated condition in stems, still undergoing elongation; yet if definitive callus on the sieve plate designates obliteration or a passive condition, as FISCHER assumes (**15,** p. 312), then these second type tubes were the only ones which were active in the peduncles of the stored fruits and senile stems mentioned above. On the other hand, if slime plug formation is a criterion of activity (**15,** p. 293), then even these were in a state of obliteration for they did not show these structures.

The most obvious conclusion which can be drawn from these various observations is that the criteria used by the early workers to designate the various stages in the development and function of these elements must be abandoned and a reinvestigation of the whole situation made, if we desire to know the true significance of sieve tubes in the physiology of the plant.

Experimental results

For a correct determination of the relation between phloem exudation and the transport of organic nutrients, quantitative data are required on

the amounts of material being moved, the linear rates of flow, and the various factors affecting this movement. In a previous publication (5) data were given which indicated that materials were being translocated by this exudation process at somewhat higher rates than are required for growth and development of fruits of the pumpkin.

At the suggestion of Professor O. F. CURTIS, of Cornell University, the effects of local chilling were studied, since his experiments (7) had shown a definite retardation of translocation resulting from this treatment. Mature cucumber plants growing in pots were used, the treatments being carried on in the greenhouse. In the first experiment, four plants growing in a large pot, and trained to a height of about 4 feet, were employed and the equipment described by CURTIS and illustrated in text figures 1 and 2 of his publication (7) was used. The cooling coil consists of short lengths of rubber tubing fastened together by small glass U-tubes, a series of several lengths being bound around the stem and insulated from the air by covering with cotton.

In these experiments two stems were chilled and two were left unchilled. After collecting the exudate, the coils were removed and placed on the latter two plants and the previously chilled plants used as checks. This alternation was repeated several times and served to eliminate individual differences in the plants. In the first set one coil was used on each stem, covering a region about 2.5 inches in length. In making the collection, the stem was cut just above the coil and the cut basal end held in a small glass tube so that the exudate collected in the bottom. At the end of one minute the tube was removed, a thin slice cut from the end of the stem, and a second one-minute collection taken in the same tube. The tubes were stoppered and weighings made as soon as the samples were all taken. Two samples from chilled and two from unchilled plants were taken in one series. When all four were collected, a small portion of the end of each stem was cut off and taken to the laboratory for sectioning. These sections, mounted fresh in tap water, were placed in a microprojector and the enlarged image outlined on paper. The paper pattern was cut out and measurements made by weighing the areas and comparing with a standard area. The results are given in table I.

In order to test the effect of chilling upon the portion of the plant below the chilled region, sampling was continued on two plants, collections being made below the chilled region immediately following those taken in this region. This required sampling for 12 minutes, and two other plants, one normal and one chilled, were used to check upon the effect of such extended exudation. Table II summarizes the results obtained.

This experiment indicates that the effect of chilling is localized and that exudation from cuts made below the chilled portion of a stem are normal.

TABLE I

RATE OF FLOW OF EXUDATE FROM CHILLED AND NORMAL PLANTS

		Normal				Chilled			
Date	Time	Exudate fresh wt.	Phloem area	Linear rate	Average rate	Exudate fresh wt.	Phloem area	Linear rate	Average rate
	min.	*gm.*	*sq. mm.*	*cm. per min.*	*cm. per min.*	*gm.*	*sq. mm.*	*cm. per min.*	*cm. per min.*
Oct. 1	0–2	0.0256	0.508	2.52	1.77	0.0321	0.777	2.06	1.22
	2–4	0.0154		1.52		0.0137		0.88	
	4–6	0.0130		1.28		0.0110		0.71	
Oct. 2	0–2	0.0496	0.973	2.55	1.87	0.0199	0.557	1.79	1.20
	2–4	0.0342		1.76		0.0121		1.09	
	4–6	0.0256		1.31		0.0080		0.72	
Oct. 2	0–2	0.0432	0.858	2.52	1.65	0.0091	0.563	0.81	0.83
	2–4	0.0294		1.71		0.0100		0.89	
	4–6	0.0123		0.72		0.0088		0.78	
Oct. 2	0–2	0.0601	0.973	3.08	2.14	0.0113	0.383	1.47	1.13
	2–4	0.0378		1.94		0.0083		1.08	
	4–6	0.0271		1.39		0.0065		0.85	
Oct. 3	0–2	0.0436	1.088	2.00	1.56	0.0228	0.624	1.83	1.12
	2–4	0.0322		1.48		0.0111		0.89	
	4–6	0.0260		1.19		0.0082		0.66	
Oct. 4	0–2	0.0298	0.672	2.52	1.60	0.0278	1.246	1.11	0.76
	2–4	0.0191		1.42		0.0176		0.70	
	4–6	0.0158		1.17		0.0118		0.47	
Oct. 4	0–2	0.0325	0.592	2.74	1.81	0.0330	0.958	1.72	1.09
	2–4	0.0185		1.56		0.0178		0.93	
	4–6	0.0131		1.11		0.0121		0.63	
Grand average				1.79 ± 0.050				1.05 ± 0.047	
Difference				0.74 ± 0.07					

$74 \div 1.79 \times 100 = 41.3$ per cent. decrease due to chilling. $\frac{D}{PE_D} = \frac{0.74}{0.07} = 10.6$

TABLE II

EFFECT OF LOCAL CHILLING UPON EXUDATION FROM UNCHILLED PORTION OF PLANT

DATE	TIME	TREATMENT	EXUDATE FRESH WT.	PHLOEM AREA	LINEAR RATE	AVERAGE RATE
	min.		*gm.*	*sq. mm.*	*cm. per min.*	*cm. per min.*
Oct. 3	0–2	Normal plant	0.0436	1.088	2.00	1.56
	2–4		0.0322		1.48	
	4–6		0.0260		1.19	
	6–8		0.0220		1.01	0.91
	8–10		0.0192		0.88	
	10–12		0.0182		0.83	
Oct. 3	0–2	Chilled plant	0.0228	0.624	1.83	1.12
	2–4		0.0111		0.89	
	4–6		0.0082		0.66	
	6–8		0.0081		0.65	0.57
	8–10		0.0070		0.56	
	10–12		0.0063		0.50	
Oct. 4	0–2	Collection from chilled portion	0.0278	1.246	1.11	0.76
	2–4		0.0176		0.70	
	4–6		0.0118		0.47	
	6–8	Collection below chilled portion	0.0336	1.136	1.48	1.23
	8–10		0.0259		1.14	
	10–12		0.0232		1.02	
Oct. 4	0–2	Collection from chilled portion	0.0330	0.958	1.72	1.09
	2–4		0.0178		0.93	
	4–6		0.0121		0.63	
	6–8	Collection below chilled portion	0.0378	1.135	1.61	1.21
	8–10		0.0257		1.13	
	10–12		0.0190		0.83	

During the foregoing experiments the temperature of the coils varied between 2° and 4° C., but the temperature of the greenhouse fluctuated between 20° and 30° C. Some samples were collected one morning when the temperature was 24° C. A second collection was made during the afternoon when it went down to 17° C. The results brought out the effect of cooling the whole plant as compared with local chilling and are given in table III. The three 2-minute collections are summed to save space.

These plants were mature and exudation from the tips was much more profuse than from the older stems, but upon this declining exudation rate the relative effects of cooling the whole plant as compared with local chilling are graphically impressed. With all four plants the lowest rate was measured at this time, indicating that a drop of 10° in the temperature of the total length of the conducting channels has a more profound effect than

TABLE III

EFFECT OF COOLING THE ENTIRE PLANT COMPARED WITH THAT OF LOCAL CHILLING

DATE	TIME	TREATMENT	TEMPERATURE OF GREENHOUSE CHILLING COILS		EXUDATE FRESH WEIGHT	PHLOEM AREA	LINEAR RATE
	min.				*gm.*	*sq. mm.*	*cm. per min.*
Oct. 20	0–6	Plant no. 1 chilled	20°–25°	1°–2°	0.0506	0.764	1.11
A.M. 21	0–6	" " normal	24°		0.0470	0.805	0.97
P.M. 21	0–6	" " chilled	17°	2°–3°	0.0140	0.761	0.23
A.M. 21	0–6	" " normal	26°		0.0452	0.980	0.77
Oct. 20	0–6	Plant no. 2 chilled	20°–25°	1°–2°	0.0487	1.067	0.76
A.M. 21	0–6	" " normal	24°		0.0744	0.914	1.36
P.M. 21	0–6	" " chilled	17°	2°–3°	0.0272	0.845	0.54
A.M. 22	0–6	" " normal	26°		0.0661	0.825	1.34
Oct. 20	0–6	Plant no. 3 normal	20°–25°		0.0653	0.672	1.62
A.M. 21	0–6	" " chilled	24°	3°–4°	0.0347	0.645	0.89
P.M. 21	0–6	" " normal	17°		0.0056	0.702	0.13
A.M. 22	0–6	" " normal	26°		0.0522	0.821	1.06
Oct. 20	0–6	Plant no. 4 normal	20°–25°		0.0499	0.814	1.02
A.M. 21	0–6	" " chilled	24°	3°–4°	0.0224	0.726	0.51
P.M. 21	0–6	" " normal	17°		0.0100	0.662	0.25
A.M. 22	0–6	" " normal	26°		0.0311	0.786	0.66

chilling a much shorter region. This result is to be expected, since in viscous flow the resistance is directly proportional to the length of the conducting element.

Realizing the seriousness of fluctuating external temperature upon exudation rates, the experiment reported in table I was repeated, an attempt being made to maintain the greenhouse temperature at 20° C. or slightly above. In this set, two chilling coils were used on each stem covering a region about 5 inches in length. At the time the collections were made the upper coil was removed and the initial cut was made in the center of the region which it covered. The results are summarized in table IV.

These results show definitely that localized chilling hinders exudation, although with the length of stems used it was not stopped. CURTIS found with his bean plants that chilling the petioles "stopped or greatly retarded" the removal of carbohydrates (7). With the type of treatment used here, exudation was retarded only about 50 per cent. There are two possible reasons, however, for this difference in results. In the first place he chilled a much greater proportion of the total stem length of his plants, and since viscous flow is hindered in direct proportion to the length of the conducting

TABLE IV

RATE OF FLOW OF EXUDATE FROM CHILLED AND NORMAL PLANTS; CHILLING COIL TEMPERATURE, 2°–4° C.

		Normal					Chilled				
Date	Time	Plant number Greenhouse temperature	Fresh weight	Phloem area	Linear rate	Average rate	Plant number Greenhouse temperature	Fresh weight	Phloem area	Linear rate	Average rate
	min.		*gm.*	*sq. mm.*	*cm. per min.*	*cm. per min.*		*gm.*	*sq. mm.*	*cm. per min.*	*cm. pr. min.*
Oct. 22	0–2	7	0.0452		3.98		5	0.0150		1.53	
	2–4		0.0241	0.571	2.12	2.62		0.0061	0.488	0.62	0.81
	4–6	22°	0.0203		1.79		22°	0.0026		0.26	
Oct. 22	0–2	8	0.0438		2.83		6	0.0168		1.24	
	2–4		0.0336	0.775	2.17	2.28		0.0066	0.674	0.49	0.64
	4–6	22°	0.0285		1.84		22°	0.0023		0.17	
Oct. 23	0–2	5	0.0354		2.24		7	0.0180		1.69	
	2–4		0.0203	0.788	1.29	1.48		0.0113	0.530	1.07	1.18
	4–6	23°	0.0143		0.91		23°	0.0084		0.79	
Oct. 23	0–2	6	0.0195		1.34		8	0.0228		1.26	
	2–4		0.0117	0.726	0.81	0.99		0.0166	0.906	0.92	0.94
	4–6	23°	0.0119		0.82		23°	0.0115		0.63	
Oct. 24 A.M.	0–2	7	0.0315		2.09		5	0.0153		0.80	
	2–4		0.0191	0.752	1.27	1.47		0.0059	0.952	0.31	0.49
	4–6	24°	0.0155		1.03		24°	0.0066		0.35	
Oct. 24 A.M.	0–2	8	0.0403		1.83		6	0.0129		0.71	
	2–4		0.0264	1.101	1.20	1.41		0.0077	0.913	0.42	0.54
	4–6	24°	0.0266		1.21		24°	0.0088		0.48	

TABLE IV.—(*Continued*)

		Normal					Chilled				
Date	Time	Plant number Greenhouse temperature	Fresh weight	Phloem area	Linear rate	Average rate	Plant number Greenhouse temperature	Fresh weight	Phloem area	Linear rate	Average rate
	min.		*gm.*	*sq. mm.*	*cm. per min.*	*cm. per min.*		*gm.*	*sq. mm.*	*cm. per min.*	*cm. per min.*
Oct.	0–2	5	0.0244		1.34		7	0.0137		0.97	
24	2–4		0.0146	0.921	0.80	0.92		0.0104	0.704	0.74	0.74
P.M.	4–6	24°	0.0118		0.65		24°	0.0071		0.50	
Oct.	0–2	6	0.0238		1.34		8	0.0227		0.78	
24	2–4		0.0199	1.054	0.94	1.10		0.0114	1.454	0.39	0.58
P.M.	4–6	24°	0.0217		1.03		24°	0.0162		0.56	
Oct.	0–2	7	0.0386		2.30		5	0.0126		0.65	
25	2–4		0.0240	0.840	1.43	1.57		0.0080	0.977	0.41	0.45
A.M.	4–6	21°	0.0167		0.99		21°	0.0059		0.30	
Oct.	0–2	8	0.0517		1.55		6	0.0071		0.31	
25	2–4		0.0303	1.673	0.91	1.03		0.0043	1.145	0.19	0.22
A.M.	4–6	21°	0.0215		0.64		21°	0.0034		0.15	
Grand average						1.49 ± 0.12					0.66 ± 0.06

Difference = 1.49 − 0.66 = 0.83 ± 0.13.

0.83 ÷ 1.49 × 100 = 55.7 per cent. decrease due to chilling.

$\frac{D}{PE_D} = 6.38$.

element and also in direct proportion to the viscosity of the solution, greater retardation was to be expected. In the second place he was measuring normal flow while in this case a rate of three or more times that of normal transport was being dealt with. The resistance in a closed system might be great enough practically to stop the flow, but if the length of the pressure gradient were suddenly shortened by cutting, rapid flow would occur until equilibrium was reached.

It seems justifiable, in the light of these experiments, to conclude that the effect of local chilling upon exudation is due to the change in viscosity of the solution of nutrients; and while protoplasmic streaming may be stopped owing to a similar increase in viscosity, it does not necessarily follow that a more direct relationship exists between protoplasmic streaming and longitudinal transport of solutes.

In order to see whether local chilling had any effect upon the composition of the exudate, some of the samples were dried to constant weight and the dry weights calculated as percentage of fresh weight. Table V shows that no significant difference exists between these samples.

TABLE V

DRY WEIGHT OF EXUDATE FROM CUCUMBER STEMS

DATE	STEM	NORMAL			CHILLED		
		TIME (MIN.)			TIME (MIN.)		
		0–2	2–4	4–6	0–2	2–4	4–6
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Sept. 30	1	8.6	7.6	6.6	10.5	9.1	7.5
	2	8.3	6.9	5.8	9.0	7.9	9.3
	3	10.6	11.0	7.8	8.8	7.5	6.5
Oct. 1	4	8.8	6.4	7.5	8.5	6.4	6.0
Average		9.1	8.0	7.0	9.2	7.8	7.4

The next set of experiments was designed to show the relation between the composition of the exudate, the composition of the fruits, and the rate of development of the fruits. On October 3, several young fruits were tagged and two were collected, the exudate from the stem attached to the plant being taken at the same time. The results are shown in table VI.

The marked cucumbers were collected on October 9, 15, and 30. The average weights on the two small ones are included in table VII to give the complete series.

TABLE VI

FRESH WEIGHT AND DRY WEIGHT OF FRUITS AND EXUDATE FROM CUCUMBER PLANT; EXUDATION RATE FROM PLANT

DATE AND PLANT	TIME	EXUDATE						FRUIT		
		FRESH WEIGHT	DRY WEIGHT	DRY PERCENTAGE OF FRESH	PHLOEM AREA	LINEAR RATE	AVERAGE RATE	FRESH WEIGHT	DRY WEIGHT	DRY PERCENTAGE OF FRESH
	min.	*gm.*	*gm.*	*per cent.*	*sq. mm.*	*cm. per min.*	*cm. per min.*	*gm.*	*gm.*	*per cent.*
Oct. 3, Plant no. 3	0–2	0.0535	0.0051	9.5		4.33				
	2–4	0.0500	0.0037	7.4	0.617	4.05	3.72	0.930	0.0693	7.45
	4–6	0.0341	0.0021	6.2		2.76				
Oct. 3, Plant no. 4	0–2	0.0527	0.0044	8.4		4.65				
	2–4	0.0314	0.0022	7.0	0.567	2.74	3.19	0.789	0.0623	7.90
	4–6	0.0246	0.0013	5.3		2.17				

TABLE VII

COMPOSITION OF CUCUMBERS AND INCREASE IN WEIGHT WITH DEVELOPMENT; EXUDATE FROM PLANT

Date	Time	Exudate					Fruit		
		Fresh weight	Dry weight	Dry percentage of fresh	Phloem area	Linear rate	Fresh weight	Dry weight	Dry percentage fresh
	min.	*gm.*	*gm.*	*per cent.*	*sq. mm.*	*cm. per min.*	*gm.*	*gm.*	*per cent.*
Oct. 3	0–2	0.0531	0.0043	8.1	0.592	4.50	0.86	0.066	7.7
" 9	0–2	0.0597	0.0046	7.7	0.540	5.50	8.42	0.453	5.4
" 15	0–2	0.0813	0.0053	6.5	0.964	4.30	72.00	2.610	3.6
" 30	0–2	0.0859	0.0053	6.2	1.395	3.07	138.50	5.740	4.1

It is evident that the exudate is consistently higher in dry weight than the fruits, and that the difference increases with development of the fruits.

Two interesting calculations may be made from these data. Between October 9 and 15 the fruits gained at a rate of $2.610 - 0.453 \div 6 = 0.36$ gm. per day in dry weight. The sap at this time had a dry weight percentage of 6.5. Then $0.36 \div 0.065 = 5.5$ gm. of fresh sap required daily. But $72.00 - 8.42 \div 6 = 10.6$ gm. daily increase in fresh weight. The fruit, therefore, was requiring 5.1 gm. of water daily in order to maintain growth. Dividing 5.5 by 0.00964 cm.^{2} we find a rate of flow of 570.5 cm. per day, or 0.396 cm. per minute for the sap. But the recorded average rate of exudation is 4.50 cm. per minute, or approximately eleven times the normal rate of flow.

If a similar calculation is made for the rate of flow of sap required to develop the full-sized cucumber, a value is obtained which approximates very closely that of 0.292 cm. per minute previously obtained for the pumpkin (5). $5.740 - 0.066 \div 0.065 \div .00964 \div (24 \times 60) = 0.235$ cm. per minute average rate of flow.

TABLE VIII

FRESH AND DRY WEIGHTS ON FRUITS AND EXUDATES OF CUCUMBER PLANTS; SAP COLLECTED FROM PLANT END

No.	Sap					Fruit		
	Fresh weight	Dry weight	Dry percentage of fresh	Phloem area	Linear rate	Fresh weight	Dry weight	Dry percentage fresh
	gm.	*gm.*	*per cent.*	*sq. mm.*	*cm. per min.*	*gm.*	*gm.*	*per cent.*
1	0.1033	0.0081	7.8	0.860	6.00	251.6	9.72	3.9
2	0.1020	0.0080	7.8	1.100	4.63	121.6	5.24	4.3
3	0.1390	0.0116	8.4	1.180	5.88	121.7	4.91	4.0
4	0.0877	0.0067	7.6	0.936	4.69	187.4	7.20	3.9
5	0.0843	0.0066	7.8	0.692	6.10	93.0	3.45	3.7
6	0.0800	0.0052	6.5	1.100	3.64	116.0	3.97	3.4
7	0.1125	0.0090	8.0	1.225	4.59	183.5	7.15	3.9
8	0.0945	0.0071	7.5	0.548	8.62	87.5	3.15	3.6
9	0.0813	0.0053	6.5	0.964	4.23	72.0	2.61	3.6
10	0.0763	0.0061	8.0	0.926	4.12	35.5	1.57	4.4
Total	0.9609	0.0737	Av. 7.7	9.531	Av. 5.05	1269.8	48.97	Av. 3.9

On October 15 a series of ten fruits, ranging from half-grown to full-grown, was collected and their fresh and dry weights along with similar data on the exudate were obtained (table VIII). The exudate was collected for two successive one-minute periods. The figures of this table show that the cucumber maintains about the same dry weight composition during the later period of its enlargement, and that this is approximately one-half that of the sap exuded from the cut peduncle. The rate of exudation is extremely high, indicating low resistance to flow into the fruit.

This study was carried further by making dry weight determinations on fruits, stems, leaves, and on the exudate from the stem and fruit (table IX).

TABLE IX

DRY WEIGHT COMPOSITION OF CUCUMBER PLANT

WEIGHT	STEM		PETIOLES	LEAVES	FRUIT	EXUDATE	
	ABOVE FRUIT	BELOW FRUIT				FRUIT END	STEM END
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Fresh	4.0520	5.5338	4.7839	7.7118	127.50	0.0341	0.0285
Dry	0.3518	0.5221	0.3648	1.0835	4.9750	0.0041	0.0031
Dry percentage of fresh	8.67	9.42	7.64	14.10	3.90	12.00	10.90

The exudate from the peduncle of the fruit was collected for a period of 16 minutes and fresh and dry weights determined. Cross-sections were made of the initial and final areas of the peduncle, and areas interpolated between these values used to determine exudation rates (table X). A thin section was cut from the end of the peduncle each minute during the collection.

It will be seen from the data of table X that there is no great difference between the rate of exudation and dry weight composition of the exudate from the fruit and from the plant. The decrease in rate and dry weight of the exudate with time is to be expected, and points to an osmotic system regaining equilibrium after being thrown out of balance by suddenly releasing the internal pressure.

Data were collected on the stored fruits previously mentioned and are given in table XI.

Exudation from these stored fruits was not maintained as in the fresh ones. The xylem was full of tyloses and had lost the water from many of its larger vessels, so that the main source of the exudate was the more or

TABLE X

RATE AND COMPOSITION OF EXUDATE FROM CUCUMBER STEM AND FRUIT

	Stem	Fruit										
Time (min.)	0–1	0–1	1–2	2–3	3–4	4–5	5–6	6–8	8–10	10–12	12–14	14–16
Fresh weight	0.0285	0.0341	0.0393	0.0341	0.0331	0.0302	0.0215	0.0369	0.0280	0.0314	0.0343	0.0239
Dry weight.....	0.0031	0.0041	0.0045	0.0038	0.0033	0.0027	0.0021	0.0025	0.0018	0.0018	0.0017	0.0010
Dry percentage of fresh	10.9	12.0	11.4	11.1	9.9	9.0	9.8	6.8	6.4	5.7	5.0	4.2
Phloem area, sq. mm.	0.727	0.727	0.800	0.868	0.937	1.005	1.072	1.205	1.341	1.466	1.610	1.738
Linear rate, cm. per min.	3.92	4.70	4.90	3.92	3.45	3.00	2.00	1.53	1.04	1.07	1.06	0.69

TABLE XI

DRY WEIGHT COMPOSITION AND EXUDATION FROM STORED FRUITS

TIME	EXUDATE					FRUIT		
	FRESH WEIGHT	DRY WEIGHT	DRY PERCENTAGE OF FRESH	PHLOEM AREA	LINEAR RATE	FRESH WEIGHT	DRY WEIGHT	DRY PERCENTAGE OF FRESH
min.	*gm.*	*gm.*	*per cent.*	*sq. mm.*	*cm. per min.*	*gm.*	*gm.*	*per cent.*
0–2	0.2037	0.0193	9.4	1.77	5.7	173.0	7.00	4.05
						225.5	9.12	4.04

less elastic turgid phloem; as soon as this became emptied, flow rapidly decreased.

It seemed desirable to know the osmotic concentration of this phloem exudate, since the pressures developed depend so largely upon it. As only small amounts of the material were available, the vapor pressure method suggested by BARGER (**1**) and used on plant sap by HALKET (**17**) was used. The first determinations were made at Columbus, Ohio, on *Echinocystis lobata*. All determinations were run in duplicate. The results are shown in table XII.

TABLE XII

OSMOTIC PRESSURE DETERMINATIONS ON PHLOEM EXUDATE OF *Echinocystis lobata*

	JULY 3, 2:00 P.M.	JULY 7, 8:30 A.M.	JULY 8, 1:15 P.M.	JULY 8, 4:45 P.M.
Concentration, molar.	0.33	0.30	0.40	0.40
Osmotic pressure at 25° C. atm.	8.5	7.7	10.3	10.3
Dry weight, per cent.	.....	10.2	7.6	8.0

The concentrations recorded in table XII indicate rather high pressures. Those given were interpolated from a concentration : osmotic pressure curve constructed from the data on sucrose given in the LANDOLT-BÖRNSTEIN tables. Since sucrose solutions were used in the determinations, these should be approximately correct. Average molecular weights calculated from these values range between 200 and 400.

More extended observations have been made on exudates from cucumber and pumpkin, samples being taken from different positions on the plants (table XIII). From these figures it may be seen that there is a consis-

TABLE XIII

OSMOTIC CONCENTRATIONS AND EQUIVALENT PRESSURES AT 25° C. OF PHLOEM EXUDATES OF CUCUMBER AND PUMPKIN

PLANT	CONCENTRATION AND OSMOTIC PRESSURE AT HEIGHT AT WHICH SAMPLE WAS TAKEN				
		9′6″	6′	3′	4″
No. 1 pumpkin, 10′ in height*	Concentration, molar.	0.31	0.25	0.26	0.23
	Osmotic pressure, atm.	8.0	6.2	6.3	6.0
		7′6″	4′		4″
No. 2 pumpkin, 8′ in height*	Concentration, molar.	0.35	0.32		0.21
	Osmotic pressure, atm.	9.0	8.2		5.4
		3′6″	2′		4″
No. 3 cucumber, 4′ in height*	Concentration, molar.	0.31	0.29		0.23
	Osmotic pressure, atm.	8.0	7.4		6.0
		5′6″	4′6″	3′	4″
No. 4 cucumber, 6′ in height*	Concentration, molar.	0.29	0.28	0.27	0.24
	Osmotic pressure, atm.	7.5	7.2	7.0	6.2
		5′6″	3′		4″
No. 5 cucumber, 6′ in height*	Concentration, molar.	0.30	0.30		0.22
	Osmotic pressure, atm.	7.7	7.7		5.7

* Plants growing in pots in the greenhouse and trained vertically on strings.

tent gradient in osmotic concentration in the exudate taken from these plants, the highest concentration coming from the region behind the zone of elongation. This shows that, at least for these plants, a concentration gradient actually exists in the conducting tracts, a relation which has been inferred from analytical data in many cases (**26, 27, 5**). The samples were very small, so that this can hardly be a case of dilution such as occurs when prolonged exudation is induced by repeated cutting of the stem.

Discussion

The possibility of a relationship between phloem exudation and translocation is obvious. The analyses of ZACHARIAS (**42**) and KRAUS (**23**) indicate that the exudate from cucurbits contains sugars, organic nitrogen compounds, potash, and phosphorus. In their late publication on translocation, MASON and MASKELL (**28**) show that these are the four types of material which accumulate above a ring in the cotton plant, and that they correspond roughly in relative amounts to those found in the above mentioned analyses.

Experiments with prolonged exudation prove that these materials move over long distances in a relatively short time. Osmotic pressure determinations indicate the sources of pressure, and dissection studies denote that

the pressure is acting through the total phloem since exudation is as rapid from a tangential cut as from a transverse one. The evidence points toward the type of mechanism previously proposed (5), and the most serious remaining problem is that of locating the channels of transport.

Previous workers have agreed fairly well upon the sieve tubes, yet it remains to be proved that they serve as conducting vessels. Many of the structures which have been cited as evidence relating to flow through these cells, that is, slime accumulations, slime bubbles or droplets, and definitive callus, can hardly justify the significance given them. The median nodules and protoplasm in the pores of the sieve plates, on the other hand, should render these structures relatively impervious to rapid mass flow; yet MÜNCH mentions exudation from the sugar pine, where they undoubtedly exist. The variety of conditions under which exudation occurs, from young growing tips lacking differentiated sieve tubes to stored fruits where the sieve plates are covered with definitive callus or the tubes filled with a dense granular content, leaves but one obvious possibility. The only continuous phase occurring under all of these conditions and occupying a reasonable volume is the cell wall. It is highly hydrated, readily permeable, and continuous throughout the plant.

Studies with polarized light show the phloem walls to be doubly refractive, indicating a certain regularity of structure; and measurements of the relative lateral and longitudinal shrinkage with dehydration (5) suggest the models of SPONSLER and DORE (39). Resistance to diffusion by the walls is slight compared with that of active protoplasm, as shown by many plasmolysis tests, but the resistance to a rapid flow of solution remains to be measured.

In certain border-line cases the distinctions between diffusional movement and mass flow break down, and in this limiting condition flow seems to consist of a movement of nutrients along a diffusion gradient accompanied by movement of water along a pressure gradient. The gradient of water may be immensely steepened by increasing the pressure. From the experiments on continued exudation, it seems possible that this accelerated flow of water may carry solutes with it at a higher rate than that of natural diffusion, and so function effectively in translocation.

The rates and pressures presented in the previous section may be used to test the various theories on translocation as to their ability to satisfy requirements. Previous work (5) has indicated rates of linear displacement of the total phloem volume of about 0.3 cm. per minute in the stolon of the developing potato tuber and peduncle of the pumpkin. In table VII of the previous section, data are given which permit the calculation of rates, and these turned out to be approximately 0.4 cm. per minute during maximum growth and an average rate of nearly 0.3 cm. per minute

throughout the growth period. The rates of flow measured in the exudation studies are much greater than this. Dividing the total fresh weight of exudate in table VIII by the total phloem area, an average rate of 5.0 cm. per minute is obtained.

If this rate is used in the POISEUILLE formula and the proper values inserted for the other factors, the pressure necessary to cause this flow through the sieve tubes may be determined:

$$R_1 = \frac{pr^2}{8l\eta}$$

where

R_1 = linear velocity of flow in cm. per sec.
p = pressure in dynes per cm.^{2}
r = radius of the capillary.
l = length of the capillary.
η = viscosity of the flowing solution.

Using previously determined values (5), the sieve tubes occupy about 20 per cent. of the phloem and the pores about 8.0 per cent. of the sieve plates, or 1.6 per cent. of the total phloem. Then R_1 would be 5.2 cm. per sec. through the pores of the sieve plate. The radius of the pores is 1μ or less and the length would be about 2 per cent. of the mean length of 50 cm., or 1 cm. The viscosity would not be less than that for a 10 per cent. sucrose solution, or 0. 015.

Then

$$p = \frac{8 \times 1 \times 0.015 \times 5.2}{1 \times 10^{-8}} = 62.5 \times 10^6 \text{ dynes},$$

or 61.8 atmospheres. Since an average of the values given in table VIII indicates that only seven atmospheres are available, the exudate collected in these experiments could not have flowed through the perforations in the sieve plates even if they were free of the protoplasmic strands which traverse them.

Considering next the protoplasmic streaming hypothesis, we can see from figure 29 that the protoplasmic lining of the cell walls is very thin. If the walls occupy 30 per cent. of the phloem area (5), then the protoplasm must occupy about 20 per cent. of this, or 6 per cent.; and since only 50 per cent. of this is streaming in the proper direction at any one time, then 3 per cent. of the total phloem content is available to accelerate movement. Since R_1 for normal sap flow is 0.3 cm. per minute or 18 cm. per hour, it would be roughly 10 per cent. of this or 1.8 cm. per hour for the dry matter being moved. Dividing 1.8 by 0.03, we find that pure solute

would have to move at a rate of 60 cm. per hour or at three times the maximum observed rate of streaming through the total space occupied by protoplasm in the phloem. Assuming that protoplasm contains about 80 per cent. water, it can readily be seen that protoplasmic streaming is unsatisfactory, for the total volume occupied by the solids of the protoplasm would need to be occupied by pure assimilate moving at fifteen times the maximum rate of streaming. Diffusion through end walls from cell to cell would be an additional difficulty, since the gradient across any one of the multitude of membranes would be almost negligible. MASON and MASKELL (**28**) find a similar disparity between streaming and conduction.

Testing next the possibility for flow through the walls, substitution in the formula gives the minimum size of capillary through which the normal rate of flow could take place.

$R_1 = 0.3 \div 0.15 \div 60 = 0.033$ cm. per sec. through the area of the phloem walls occupied by water.

$p = 7.0$ atmospheres.

$l = 50$ cm.

$\eta = 0.015$.

$$r^2 = \frac{8 \times 0.033 \times 0.015 \times 50}{7.0 \times 980 \times 1033} = 2.8 \times 10^{-8} \text{ cm.}^2$$

$r = 1.67 \times 10^{-4}$ cm. and the diameter of the capillary would be 3.3μ.

If flow were taking place between concentric cylinders, or in the limiting case, parallel planes, the formula becomes

$$d^2 = \frac{3 R_1 l \eta}{p}$$

where $2d$ = the distance between the planes. Substituting as before

$$d^2 = \frac{3 \times 0.033 \times 0.015 \times 50}{7.0 \times 980 \times 1033} = 1.04 \times 10^{-8} \text{ cm}^2.$$

Then

$$d = 1.02 \times 10^{-4} \text{ cm.}, \quad 2d = 2.04 \times 10^{-4} \text{ cm., or } 2.04 \mu.$$

Since both the diameter for the minimal capillary and the minimal distance between parallel planes are much larger than would be expected for pore spaces in a jell, it becomes evident either that flow through the walls does not obey the viscosity formula or that some other mechanism is involved.

The studies made on the penetration and accumulation of stains by sieve tubes suggest the possibility of another explanation for translocation

through the phloem. In young active sieve tubes exhibiting protoplasmic streaming, neutral red is readily accumulated but anilin blue penetrates with great difficulty. As the protoplast matures, neutral red is accumulated less and less and anilin blue penetrates more and more readily, staining slime drops, nucleus, and the callus of the sieve plate. Finally, after the slime drops and nucleus disappear, neutral red is no longer accumulated, the cells can no longer be plasmolyzed with sucrose solutions, and anilin blue penetrates readily.

Although not providing proof, these observations indicate an increasing permeability of the protoplasm of the sieve tube with maturity. If this is the case, there is the possibility that flow actually occurs partly in the walls and partly in the lumina of the sieve tubes, at least in those portions of the plant where mature sieve tubes exist. If this were the case, all that would be necessary in order that the sieve tubes show their usual characteristics is that they maintain a slight excess of turgor pressure above the hydrostatic pressure present throughout the phloem. This they apparently do, for in fresh sections of living material, although failing to plasmolyze in hypertonic sucrose solution, they appear to maintain turgor unless injured in some way in the preparation of the section.

Granting that such a combination occurs and that flow takes place partially through sieve-tube segments, it still seems improbable that the sieve plate is adapted to facilitate movement. Entry and exit would more likely take place between the protoplasmic connections and along the side walls. Certain microchemical tests indicate the presence of soluble reducing substances in phloem walls which disappear upon leaching. If leakage from sieve tubes actually occurs, an explanation of their presence is greatly simplified.

The mechanism of MÜNCH, namely, movement from living cell to living cell by way of the plasmodesma, seems adequate in the mesophyll of the leaf because of the short distances and enormous surfaces exposed. If then the solution of assimilate passes from palisade to spongy parenchyma and thence by the border parenchyma to the phloem, and there is released into cells with increasing permeability, it should slowly leak from these cells into the walls. By virtue of its osmotic activity it should continue to increase in volume until equilibrium is reached, and should flow in whichever direction the pressure gradient lies. The only other restriction would be the external limiting layer of the phloem. Thus it could flow to the growing point to supply nutrients to the developing stem, or to the root where it would nourish the meristems and supply storage organs.

Transport into the wood and out to the cortical tissues of the root appears again to be a case of movement from living cell to living cell, and wherever this type of movement occurs protoplasmic streaming should aid

materially in accelerating transfer. In longitudinal movement through the petiole and stem, however, it would seem totally inadequate, as it also would in explaining the exudation from cut stems observed in these studies.

As the mechanism pictured here is developed, it can be seen to differ radically from that of MÜNCH. The latter works between the turgor pressure of the assimilating cell and that of the utilizing cell. If this latter happened to be a meristematic cell of the growing point or cambium, it might have a higher turgor than the assimilating cell and translocation would become difficult to explain. In the mechanism herein pictured, the pressure gradient runs from the turgor of the assimilating cell to zero in the limiting case, and to a pressure approaching zero in all cases, it being assumed that the active growing or storing cell can maintain a low concentration of nutrients in its inclosing walls, leaving the water free to move where it will. This type of mechanism seems to fit more of the data and satisfy more of the requirements than many of the previously described theories. MASON and MASKELL (**28**) object to the mechanism of MÜNCH, feeling that the various substances which they have studied move independently, depending upon individual concentration gradients. When one considers the complexity of the system through which materials are moving in the mechanism being considered, it seems possible that this independence of movement may be only apparent. As the mixture of nutrients leaves the assimilating cells of the leaf, it is subject to losses all along the way from absorption by living cells. There is the possibility of increase in inorganic ions by diffusion across the cambium, as well as loss to the wood of all materials by leakage along the rays. Calcium is combined with organic acids in various regions and rendered immobile; sugar is respired at different rates in different tissues; meristematic activity occurs along the total line of transport while storage may be distinctly localized. Under this variety of conditions it would be difficult to determine the relation of any one constituent to the rate of flow or composition of the total mixture.

With the possibility of movement through both sieve-tube lumina and phloem walls, the relative importance of these depends upon the resistance which each presents to mass flow. These resistances remain to be determined by future work.

Summary

1. The theory that translocation of organic foods in plants occurs in sieve tubes and is facilitated by perforation of the end walls was developed by HARTIG, NÄGELI, VON MOHL, and other early botanists and persists as the prevailing concept today.

2. Anatomists have considered the sieve plate to be perforated as an adaptation to rapid flow and few physiologists have questioned this mechanism.

3. Protoplasmic streaming has been suggested as an accelerating mechanism in translocation in sieve tubes.

4. KUHLA, SCHMIDT, RUHLAND, BIRCH-HIRSCHFELD, DIXON, BALL, and CRAFTS have raised various points in contradiction to this classical theory.

5. Sieve tubes in cucurbits are of two types: (*a*) central tubes of the phloem and (*b*) peripheral tubes of the phloem, commissural, entocyclic, and ectocyclic tubes.

6. Type *a* tubes have short, broad elements, an internal protoplasmic mesh in mature stages, and a low coagulable content. Slime plugs and definitive callus occur under certain conditions.

7. Type *b* tubes vary greatly in length but tend to be uniform in diameter. Internal protoplasmic strands are limited to sieve plates, and in mature stages these elements have a dense granular content. Slime plugs have not been found, and definitive callus appears only after obliteration or partial collapse.

8. Slime drops occur in all young sieve tubes. The coagulable contents of type *a* tubes and the granular material in type *b* tubes seem related to distintegration of these structures.

9. Protoplasmic connections traverse the sieve plates of mature sieve tubes, connecting the successive elements. While each strand is surrounded by a callus cylinder, no central pore has been found.

10. Definitive callus is formed by elongation and lateral fusion of the callus cylinders, the protoplasmic connections being stretched in the process. No callus "plugs" capable of "stopping" flow have been found.

11. Slime plugs are caused by killing reagents or gravitation and are not related to normal sap flow.

12. Mucous droplets or bubbles occur only near cuts and they would seem to hinder mass flow.

13. Protoplasmic connections of the sieve regions are intimately related to the parietal cytoplasm and appear to have a protoplasmic content.

14. Exudation from cut stems is practically limited to the phloem, except at the growing tips.

15. Exudation from the cut peduncle of a fruit stored for six weeks was normal although the sieve tubes were heavily callused or had dense granular contents.

16. Local chilling of cucumber stems resulted in a 55.7 per cent. decrease in exudation rate. Cooling the entire plant to 17° C. reduced this rate even more. Local chilling did not affect dry weight of the exudate or exudation rate from unchilled portions.

17. Calculations indicate a sap flow in cucumber equivalent to displacement of the total phloem at a rate of 0.24 cm. per minute. Developing fruits have a dry weight of about 3.9 per cent. Exudate from these fruits has an average dry weight of 7.7 per cent. Exudation from cut peduncles is very rapid.

18. Exudates from pumpkin and cucumber have an osmotic concentration equivalent to an average pressure of 7.0 atmospheres. Concentration decreases from the tips toward the roots.

19. The only continuous permeable phase throughout the plant is the cell wall. In phloem it is highly hydrated, shows regularity of structure, and occupies about 30 per cent. of the total volume in fresh sections.

20. Calculations indicate that exudation from cut peduncles cannot be explained on the basis of mass flow through perforations in the sieve plates.

21. In view of the increasing permeability of the sieve-tube cytoplasm, as indicated by staining and plasmolysis tests, it seems possible that movement may take place partly through sieve-tube lumina and partly through phloem walls.

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EXPLANATION OF PLATES IV–IX

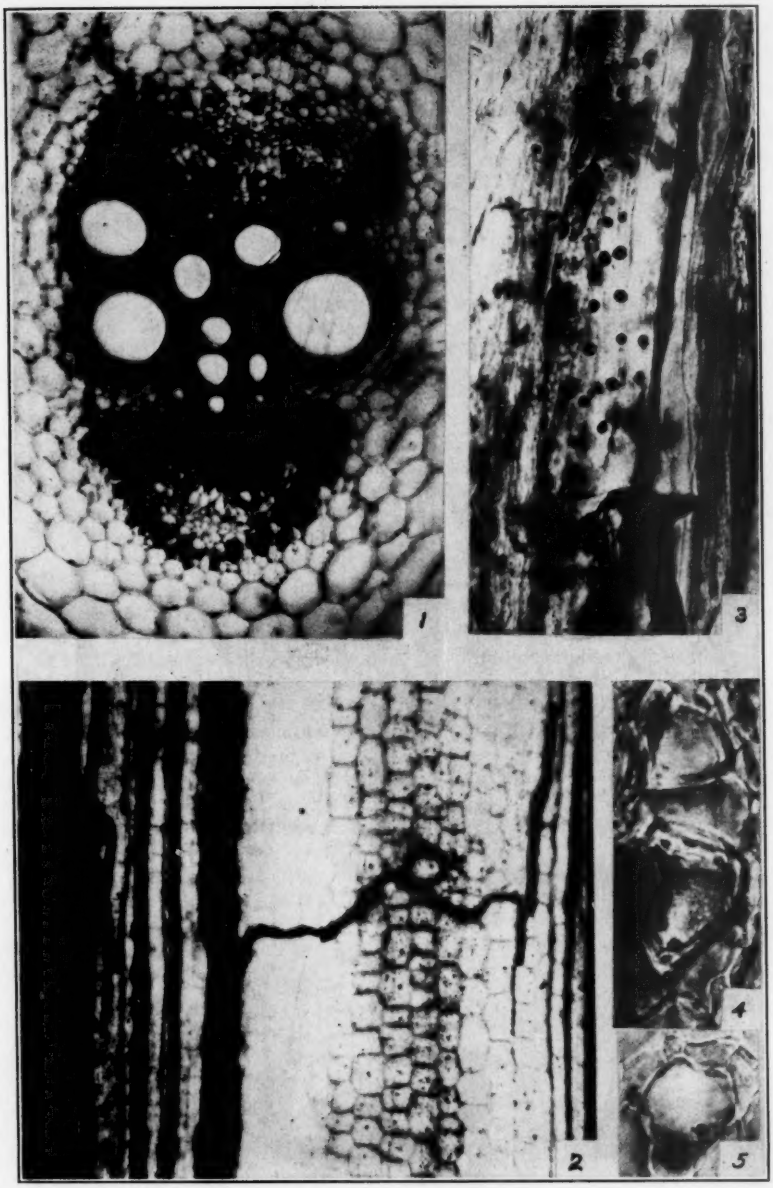
PLATE IV

FIG. 1. Transverse section of vascular bundle of pumpkin stem killed in hot water and stained with water blue. ×75.

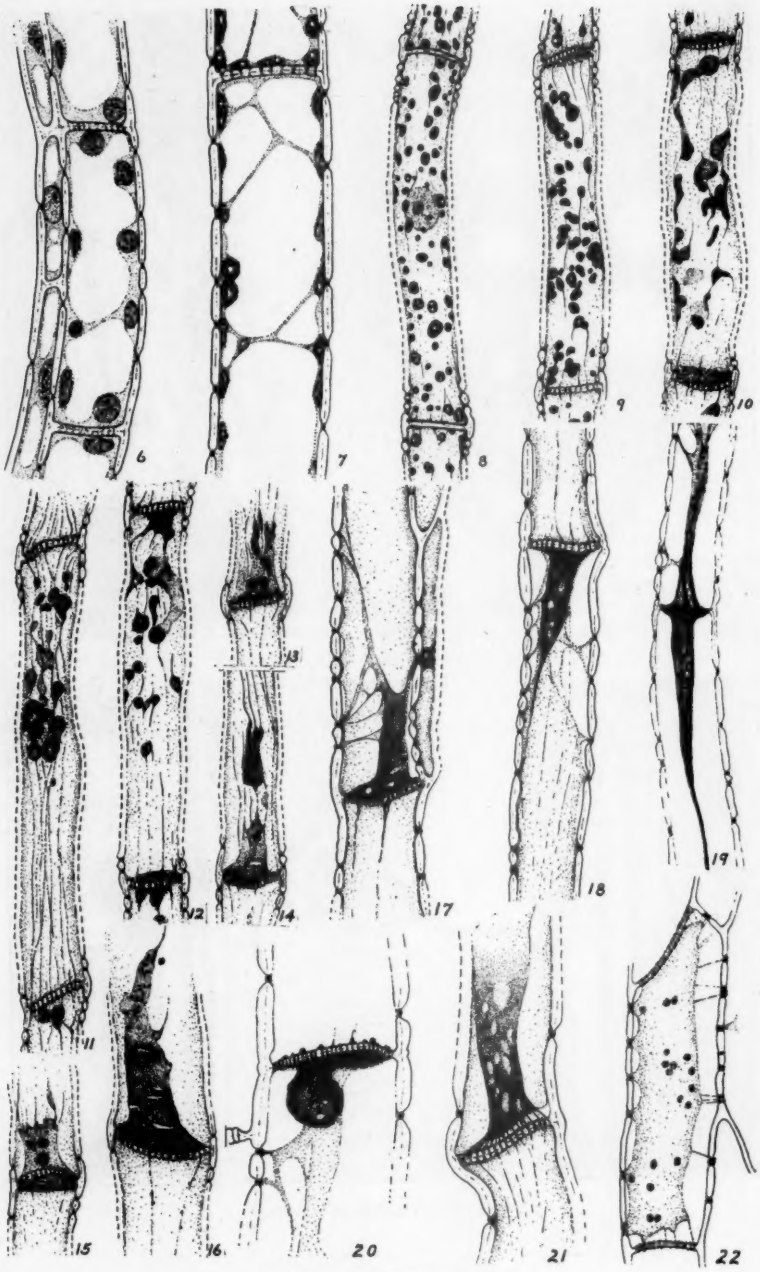
FIG. 2. Longitudinal tangential section of pumpkin stem killed in hot water and stained with water blue. Peripheral tubes of phloem on the left; commissural tube traversing parenchyma tissue, and entocyclic sieve tubes on the right. ×75.

FIG. 3. Longitudinal section of cucumber stem showing slime drops fixed in 50 per cent. alcohol mordanted with IKI solution and stained with water blue. Slime accumulation in mature sieve tube on the right. ×330.

FIGS. 4, 5. Transverse sections of cucumber (4) and pumpkin (5) stems showing slime drops in parietal protoplasmic layer. Killed in hot water fixed with alcohol and stained with water blue. ×690.



CRAFTS: TRANSLOCATION



CRAFTS: TRANSLOCATION

PLATE V

FIG. 6. Freehand drawing showing optical section through young living sieve tube and companion cell of cucumber stained with neutral red. The protoplasm was streaming and constantly changing in form. ×400.

FIG. 7. Similar illustration of more mature sieve tube. The protoplasm was showing plastic movements and strands were narrow, often stretching and breaking while the section was under observation. ×400.

FIGS. 8–12. Camera lucida drawings of sieve tubes of cucumber showing slime drops in successive stages of development. Fig. 8, young stage; fig. 12, slime drops disintegrating. The internal protoplasmic strands are threadlike in fixed material. From stems killed in hot water, fixed in 50 per cent. alcohol and stained with water blue. ×175.

FIGS. 13–15. Slime drops accumulated in sieve tubes of cucumber stems cut and placed in boiling water. The sieve plates illustrated were near the cut end and accumulation was due to the killing action of the hot water. Sections fixed in alcohol and stained with water blue. ×175.

FIG. 16. Slime accumulation in mature sieve tube of cucumber after disintegration of slime drops. Section from stem treated as in previous figures. ×175.

FIGS. 17, 18. Slime accumulation in sieve tubes of cucumber stems killed and fixed in 50 per cent. alcohol. Parts of the internal protoplasmic structure adhering to the pits in side walls. ×400.

FIGS. 19, 20. Slime accumulation in sieve tubes of cucumber stems killed and fixed in 95 per cent. alcohol. In figure 19 the parietal protoplasm has contracted and closely surrounds the coagulated slime; in figure 20 the violent contraction of the protoplasmic structures has entirely ruptured them on upper side of plate, while on lower side remnants of parietal layer remain; inner threads have contracted and are hidden within the mass of coagulated slime. Fig. 19, ×175; fig. 20, ×400.

FIG. 21. Sieve tube of pumpkin stem killed in hot water, showing protoplasmic connections drawn out by slight contraction of protoplasm. ×400.

FIG. 22. Cell of epidermis of vein from dorsal side of cucumber leaf stained with pyoktanin blue to show protoplasmic connections. Protoplast was plasmolyzed and threads remain connected to pits. ×175.

PLATE VI

FIG. 23. Sieve plate in cucumber showing protoplasmic connections as black spots surrounded by callus cylinders. ×690.

FIG. 24. Same sieve plate with focus raised to show the protoplasmic strands as light spots surrounded by the darker callus cylinders. ×690.

FIG. 25. Same sieve plate with side illumination to bring out the crater-like appearance of ends of the callus cylinders. ×690.

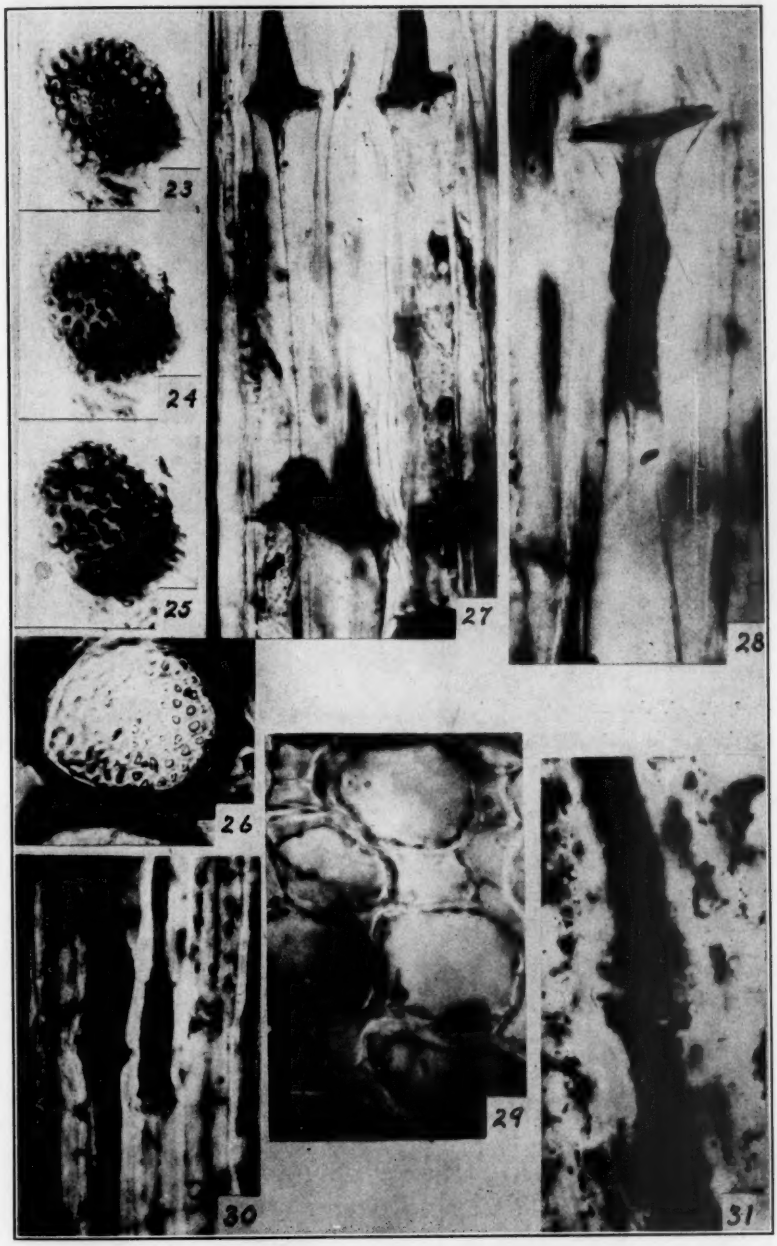
FIG. 26. Young sieve plate in pumpkin stem showing initiation of the callus cylinders surrounding protoplasmic connections. ×400.

FIGS. 27, 28. Mature sieve tubes of cucumber stem killed in 50 per cent. alcohol, showing slime accumulations, protoplasmic strings, and parietal protoplasm. Fig. 27, ×330; fig. 28, ×690.

FIG. 29. Transverse section of pumpkin stem showing thin parietal protoplasmic layer slightly plasmolyzed in places. ×540.

FIG. 30. Slime accumulations in section of pumpkin stem. The section on the slide was touched on each end with a hot needle and this figure shows the resulting slime accumulations on both sides of same sieve plate. ×690.

FIG. 31. Callused sieve plate from old pumpkin stem stained with pyoktanin blue to show protoplasmic connections. ×690.



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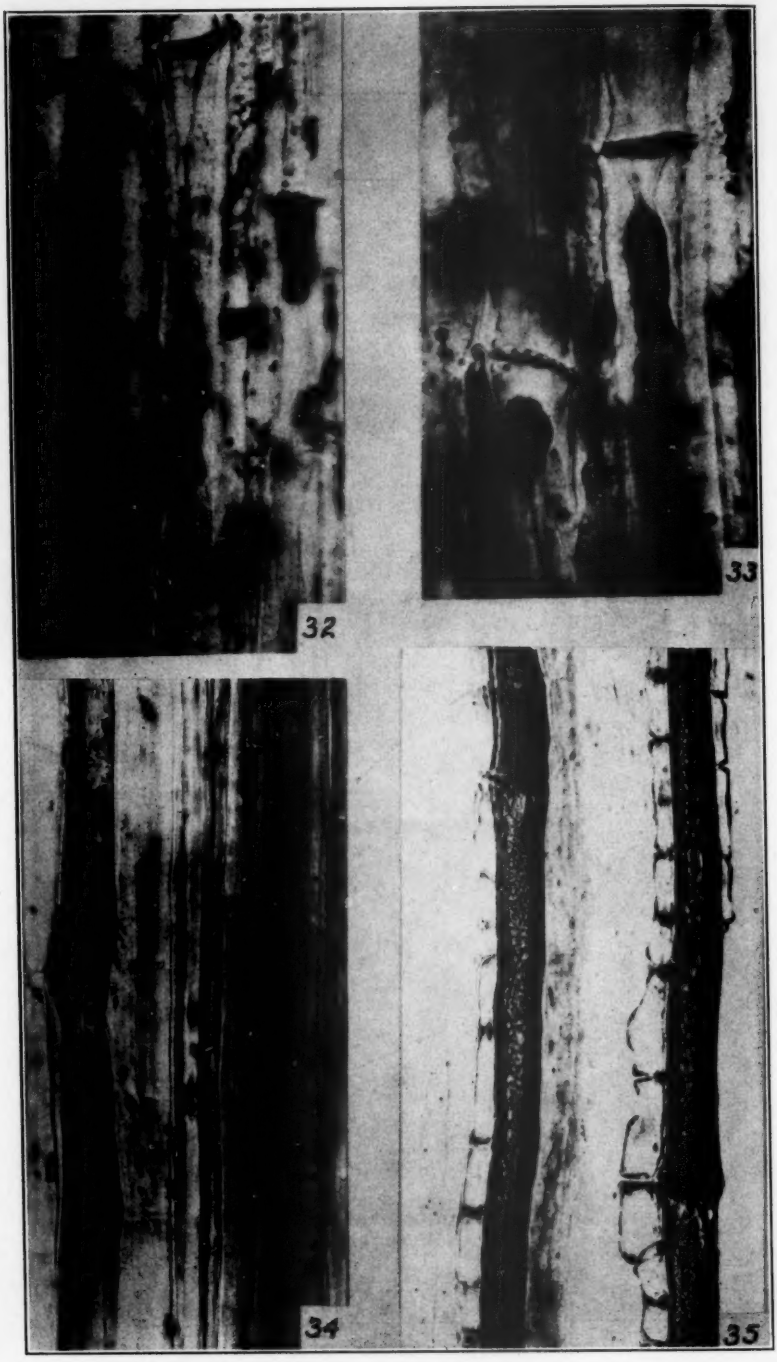

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PLATE VII

FIG. 32. Slime accumulation in cucumber stem killed and fixed in 50 per cent. alcohol. Pieces of stem about 6 mm. in length were placed in the alcohol for 30 minutes and then sectioned. In the portion midway between the two ends, slime accumulations occurred in various positions in the sieve-tube elements. At cut ends they were present on the sieve plates on the side away from the cut. ×330.

FIG. 33. Slime accumulation in section of cucumber stem treated on the slide with a hot needle, illustrating condition at a distance from the treated end. ×690.

FIG. 34. Entocyclic sieve tube of pumpkin in slime drop stage. Slight plasmolysis in the lower element brings out relation of the slime drops to the parietal protoplasmic layer. ×330.

FIG. 35. Sieve tubes at periphery of the phloem group (*cf.* with figs. 1 and 2) of same stem as shown in figure 34. These tubes are more mature but do not yet have definitive callus on their sieve plates. ×330.

PLATE VIII

FIG. 36. Pumpkin stem killed in hot water, showing densely filled peripheral sieve tube on right and central tubes with slime accumulations in center. ×150.

FIG. 37. Entocyclic sieve tube of cucumber with definitive callus on sieve plate and having cracks in the dense amorphous contents. ×410.

FIG. 38. Entocyclic sieve tube of pumpkin with amorphous mass at the sieve plate and granular content in remainder of tube. ×330.

FIG. 39. Peripheral tubes in same stem as illustrated in figure 38. Amorphous material lines the walls, leaving granular core in center of the tubes. Contents of tube on right have become completely amorphous but have not yet cracked as in figure 37. ×330.

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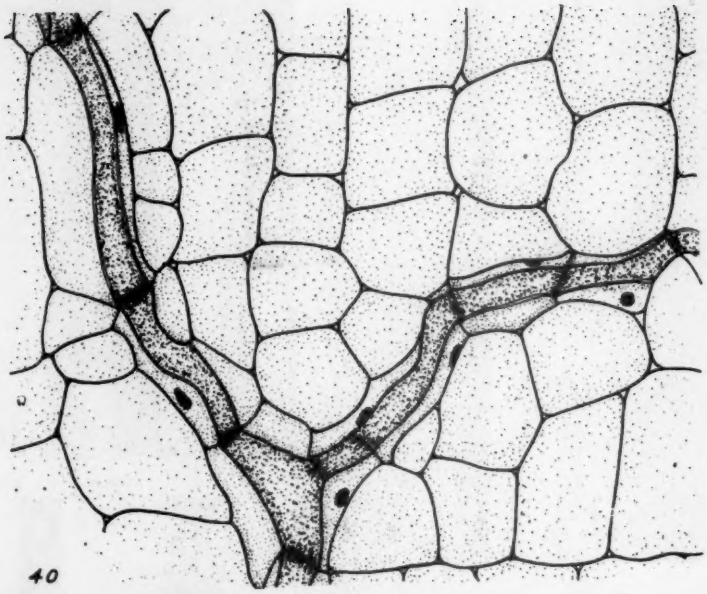

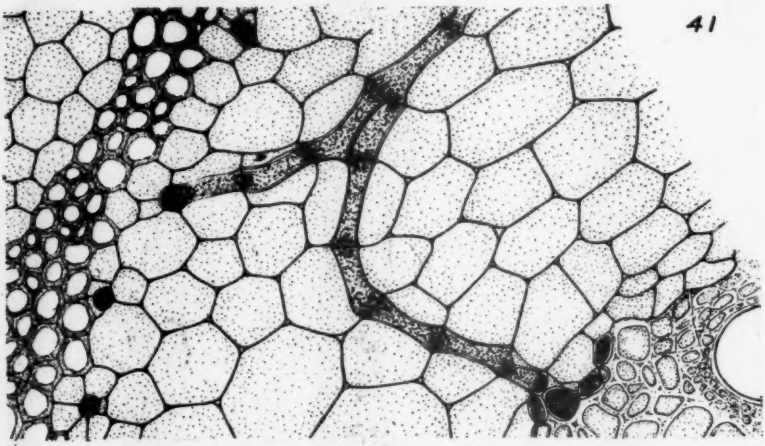

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PLATE IX

FIG. 40. Camera lucida drawing of longitudinal section of cucumber stem showing commissural sieve tubes traversing parenchyma tissue. ×96.

FIG. 41. Camera lucida drawing of transverse section of cucumber stem showing relation of commissural tubes to phloem groups and entocyclic tubes. ×96.

FACTORS AFFECTING THE DISTRIBUTION OF IRON IN PLANTS[1]

C. H. Rogers and J. W. Shive

(WITH EIGHT FIGURES)

I. Introduction

The influence of acid and alkaline reactions of the growth medium on iron relationships in plants has been known, or at least suspected, for over a century. The internal conditions of the plant in their relation to iron metabolism and chlorophyll production are not at all well understood. A number of investigations (**22, 23, 20, 18, 16, 14, 10, 3, 1**) have been carried out in which the general conclusion is that the reaction of the growth medium governs, to a large extent, the quantities of iron available to the plant. These observations are in keeping with the results obtained by Patten and Mains (**24**). Working with HCl solutions containing definite concentrations of iron, they found that, beginning with a pH of 3.5, iron was precipitated in increasing amounts up to pH 6.0, above which point practically no iron remained in solution. This was accomplished by adding, in separate experiments, ammonium hydroxide, sodium hydroxide, and hydrogen sulphide to the hydrochloric acid solutions containing definite quantities of iron. The range of iron precipitation under these conditions thus extends from pH 3.5 as the lower limit to pH 6.0 as the upper limit.

All of this work deals with the influence of the reaction of the growth medium on the availability of iron. Very little has been said regarding the possible influence of internal reactions on the availability and mobility of iron within the plant. Investigations (**21, 12, 13, 17**) in which actual quantitative measurements were employed in determining iron in the plant tissues generally indicate that plants chlorotic from lack of available iron contain as much, or more, total iron per unit weight of tissue than do normal green plants of the same species. From this it follows that certain internal conditions may render unavailable to the chlorophyllous cells the iron already in the plant.

Ingalls and Shive (**15**) found that not only does the H-ion concentration of plant tissue fluids fluctuate over a 24-hour day and night period, but that the soluble (filterable) iron content also fluctuates, and, in most cases, is directly proportional to the H-ion concentration of the respective tissue fluids. The pH of fleshy succulents fluctuates much more than does that of thin-leaved or non-succulent forms, the H-ion concentration in all

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Plant Physiology.

cases being higher at night and decreasing with increased light intensity. Plants with a pH of tissue fluids lying near or at the upper limits of iron precipitation show a much greater fluctuation of filterable iron content following variation in pH of tissue fluids than do plants with a pH near the lower limits of iron precipitation.

GUSTAFSON (**11**), using *Bryophyllum calycinum*, found a fluctuation of approximately two pH units over a 24-hour period. On the other hand, plants kept in darkness (all other factors being similar) showed very little or no fluctuation over the same 24-hour period.

AUBERT (**4**) was one of the pioneers who attempted to explain why plants were more acid at night than in the daytime. His work was done with cacti,—fleshy succulents in which there is considerable fluctuation in pH from day to night. He concluded that increased acidity at night is due to the formation of organic acids, chief of which is malic acid. The acid formation is the result of incomplete or partial oxidation of the more complex compounds. In the daytime, these acids are broken down by the sunlight: a photolytic process. SPOEHR (**29**), working with the same plant, arrived at conclusions similar to those of AUBERT. By the action of sunlight and a mercury arc lamp, he found that malic acid solutions are decomposed and the acidity of expressed cactus sap is greatly decreased. RICHARDS (**26**), also using the cactus, arrived at conclusions similar to those of AUBERT and SPOEHR. He thinks, however, that this partial oxidation, acid-forming process, goes on in the daytime as a part of natural processes, but is hidden, for the most part, by photolytic action, which breaks down the acids formed. WARMING (**30**) finds it generally true that in succulent xerophytic land plants the air-containing intercellular spaces are very narrow. In this connection he cites measurements by ALTENKIRCH. From these facts and the researches of AUBERT (**4**), WARMING thinks that this limited air passage does not allow for sufficient oxygen supply; hence the formation of acids due to partial oxidations.

II. Experimentation

A. Introductory

It has frequently been found that certain plants become chlorotic, apparently from lack of iron in the chlorophyllous cells of the leaves, although excessive quantities of iron are present in the tissues of the stems and leaves of these plants. The problem here investigated is an attempt to find in just what tissues these excessive accumulations of iron occur, and to determine why such a phenomenon occurs in some plants and not in others.

Experiments were planned whereby quantitative determinations might be made for total and soluble iron content of plants having juices with very high pH values and others having tissue fluids with very low pH values, the

plants being grown both in artificial media and in the open field. For determinations of soluble iron, the juices of the stems and leaves were extracted and filtered and the filtrates analyzed. Total iron was determined by analyses of the dried plant tissues. A study was then made of the relation between soluble and total iron content of these plants and the pH values of the extracted tissue fluids, to determine to what extent pH values of the extracted juices influenced distribution and availability of iron in the plants for chlorophyll production.

An investigation was also undertaken to determine the H-ion concentrations of the various tissues and of cells in sectional areas of the different organs; and to study the influence of pH values of the different tissues upon iron accumulations in them, and upon translocation and precipitation of iron in these plants. This investigation was carried out by means of microchemical methods and microscopical observation of sectional areas, which are described in the following pages.

B. Treatment of samples preparatory to analysis

In order to obtain comparative results, plant samples for pH and iron determinations must be representative, and must be taken at definite intervals over a period of 24 hours, since it has been shown that the pH of plant sap varies with light intensity (**11, 15**) and the soluble iron content varies with the pH (**15**). In the present series of experiments, it was decided to collect samples at 4-hour intervals, namely, at 10 A. M., 2 P. M., 6 P. M., 2 A. M., and 6 A. M. The collection of samples was always made on clear days, so that maximum differences in light intensity between day and night could be obtained.

The samples were broken or cut with a highly polished, stainless steel knife into small pieces and thoroughly mixed, so that representative samples for analyses could be obtained. This was the only time during the procedure that the material was allowed to come in contact with anything but glass surfaces. Part of the material collected at each interval was put into small glass dishes suitable for weighing. From this, dry weight and total iron determinations were made. The other part of each sample was put into glass vials, paraffined cork stoppers were inserted, and the whole plunged into an ice-salt mixture and frozen as soon as possible. After being thoroughly frozen, the material was allowed to thaw in warm water and the sap was extracted by means of a small handscrew press. Two or 3 cc. of the sap were poured into a short Pyrex tube, and electrometric pH determinations were made immediately. The remainder of the sap (at least 4 or 5 cc.) was filtered through a high-grade quantitative filter-paper. One cc. duplicate or triplicate samples of the filtered juices were measured into Pyrex digestion tubes immediately after filtration was completed. Iron

analyses of these samples were later made. Green weights were quickly obtained and recorded, using the material set aside for this determination. These procedures were accomplished as quickly as possible after sampling, in order to avoid any appreciable physical or chemical changes before determinations were completed. After weighing the green tissue, it was dried in an electric oven at 85° C. for 48 hours, and then six to eight hours at 102°. The dry weights were recorded and this material was preserved for total iron determinations. In order to calculate the amount of filterable (soluble) iron per gram of dry tissue, the number of grams of water per gram dry weight was determined, and this figure multiplied by the figure representing the amount of iron found in the 1-cc. sample of filtered juice; the resulting figure was taken to represent the amount of soluble iron per gram of dry tissue.

The material to be collected was thoroughly washed by spraying with distilled water once the day before and again three or four hours before collecting of samples began. This was done to rid the tissue, as much as possible, of dust and dirt that might have accumulated during growth of the plants.

III. Analytical methods

A. Microchemical

pH DETERMINATIONS.—In making pH determinations of cells and tissues, a series of indicator dyes recommended by CLARK (5) and SMALL (27) was used. The series consisted of the following eight dyes, listed with their color and pH ranges:

Meta Cresol Purple	Red 1.2–2.8 Yellow
LaMotte Yellow	Red 2.6–4.2 Yellow
Bromphenol Blue	Yellow 3.0–4.6 Blue
Bromcresol Green	Yellow 3.8–5.4 Blue
Methyl Red	Red 4.4–6.0 Yellow
Chlorphenol Red	Yellow 5.2–6.8 Red
Bromthymol Blue	Yellow 6.0–7.6 Blue
Phenol Red	Yellow 6.8–8.4 Red

Aqueous solutions of the alkali salts were prepared according to directions given by CLARK (5).

For making the actual pH determinations, free-hand sections of the tissues were made with a sectioning razor, the sections quickly rinsed in distilled water, and placed in spot plate depressions containing the dye solutions. A large spot plate with eight depressions was used, so that different sections of the same tissue could be put in all of the eight dyes at the same time. After standing half an hour or more, observations could be made. This was accomplished by quickly rinsing the sections in distilled water,

placing on a slide in a drop of distilled water, and immediately observing with the microscope.

IRON TESTS.—Tests for iron were made by making free-hand sections with a high quality, highly polished, stainless steel sectioning razor, quickly rinsing in distilled water, treating with a dilute (1–15) solution of hydrochloric acid, and then from 15 to 30 minutes with a 1.5 per cent. solution of potassium ferrocyanide. In the presence of iron, a blue precipitate is formed. This iron complex, commonly called Prussian blue or Berlin blue, is ferric ferrocyanide, and is formed in the presence of ferric iron. Ferrous iron may be determined by using potassium ferricyanide, but for total iron (ferrous and ferric iron) this is unnecessary, since the ferrous ferrocyanide formed in the presence of ferrocyanide and ferrous iron soon oxidizes to the ferric ferrocyanide. These tests may also be made either directly on the micro slide, or in watch glasses and then transferred to the slide. In all of the microchemical tests, the sections were handled with glass-tipped forceps.

B. MACROCHEMICAL

PH DETERMINATIONS.—pH determinations were made on about 3 cc. of extracted sap, by means of a hydrogen electrode and Type K Leeds and Northrup potentiometer. Hydrogen was bubbled through the solution until a constant potential was obtained, a procedure which usually required about 40 seconds. The electrode was cleaned and blacked frequently and checked against a standard buffer solution at frequent intervals throughout the work.

IRON ANALYSES.—Iron analyses were made according to the WONG (**31**) method, somewhat modified, which consisted of thoroughly digesting the sample with sulphuric acid, oxidizing with sodium chlorate, and adding potassium thiocyanate; after which the contents were made to a known volume and colorimetrically compared with a known standard solution. The red coloration obtained is due to the formation of ferric thiocyanate. The analyses were made in Pyrex digestion tubes, graduated to 12.5 cc. and 25 cc. Details of the method are given by INGALLS and SHIVE (**15**), and may therefore be omitted here.

The material for total iron was furnished by that used in making moisture and dry weight determinations. This material was ground to a powder with a mortar and pestle and allowed to dry for a day, first in the oven at 75° C. for six hours, and then in a desiccator. One-tenth gram samples were accurately weighed and transferred to the previously mentioned graduated Pyrex digestion tubes.

IV. Influence of H-ion concentration on soluble and total iron content

The purpose of these experiments was to study the relations of H-ion concentration to total iron and soluble iron in plants with juices having very high and very low pH reactions, and to determine whether the filterable (soluble) iron content of such plants remains fairly constant over a 24-hour day and night period under these conditions of very high and very low pH reactions. In accordance with the work of PATTEN and MAINS (**24**), in which they found that all iron was precipitated from a hydrochloric acid solution at and above a pH of 6.0, while all iron remained in solution below a pH of 3.5, it might be inferred that plant juices with a pH above 6.0 contain no soluble iron, and those with a pH below 3.5 contain iron in the soluble form only. From the data presented in these experiments, it becomes apparent that the results obtained by PATTEN and MAINS with hydrochloric acid solutions cannot be applied to extracted plant juices.

By determining the pH values of the expressed juices of a number of species, it was found that the juices of *Oxalis repens* and *Rumex acetosella* had the lowest pH values of any species tested, while *Solanum tuberosum* and *Glycine max* juices had rather high pH values. These plants were grown in ordinary wooden flats containing a well composted and thoroughly mixed soil.

Samples of the plants were collected at 4-hour intervals over a 24-hour (day and night) period, and the juices extracted as previously described. The results of the pH and iron determinations of the juices of these plants are given in tables I and II, and the averages of these over the 24-hour period are given in table III. The data from tables I and II are illustrated graphically in figures 1 and 2 respectively.

In preparing the graphs illustrating the relations of light intensity, H-ion concentration, and soluble iron content, both iron and pH values were plotted on the same graph, using a common abscissa but different ordinates. For purposes of clearer illustration, the values for soluble iron were arranged in the inverse or descending order, since these values decrease with increasing pH values.

It has been shown by INGALLS and SHIVE (**15**), and it might well be expected, that in such a plant as *Bryophyllum*, where the pH of the plant juice fluctuates widely from near the lower limits to the upper limits of iron precipitation (pH 3.5–6.0), according to PATTEN and MAINS, the soluble iron content might also vary over a wide range during consecutive periods of high and low light intensity; and it has indeed been found that the higher the light intensity and the higher the pH of the plant tissue

fluids, the lower is the soluble iron content. If the results of PATTEN and MAINS could apply to plant juices, it might then be expected that in plants which show fluctuating pH values, either entirely above the extreme upper limit (pH 6.0) of iron precipitation or entirely below the extreme lower limit (pH 3.5), there should be little or no variation in the soluble iron content with variation in light intensity and with resulting variations in pH values of tissue fluids. Again, from the experimental results presented here, it will be seen that such values do not hold true in the case of extracted plant juices.

INGALLS and SHIVE (**15**), working with nine different species with pH values ranging from 4.04 to 6.1, found the soluble iron content to vary in an inverse relation with the pH values; that is, decrease in pH values of plant juices is followed by a corresponding increase in soluble iron content, and *vice versa*. Some of the species showed much greater fluctuations in pH values and soluble iron content of the tissue fluids than did others, the fluctuation increasing with increased succulency of the plants.

OXALIS AND RUMEX

It will be observed from table I that fluctuations in the pH of the plant juices of *Oxalis* and *Rumex* do not vary through wide ranges with variation in light intensity from day to night. *Oxalis* shows a range in pH

TABLE I

pH, FILTERABLE AND TOTAL IRON IN *Rumex* AND *Oxalis* OVER A 24-HOUR PERIOD

SPECIES	TIME OF COLLECTING	pH	IRON (MG. PER GM. DRY WEIGHT)	
			FILTERABLE	TOTAL
			mg.	*mg.*
Oxalis (leaf)	10 A. M.	1.995	0.063	0.167
	2 P. M.	1.893	0.053	0.158
	6 P. M.	1.961	0.047	0.183
	10 P. M.	2.198	0.050	0.189
	2 A. M.	2.164	0.072	0.165
	6 A. M.	1.927	0.061	0.160
Rumex (leaf)	10 A. M.	2.502	0.092	0.194
	2 P. M.	2.603	0.072	0.194
	6 P. M.	2.671	0.075	0.196
	10 P. M.	2.570	0.077	0.206
	2 A. M.	2.520	0.092	0.191
	6 A. M.	2.468	0.093	0.200

variation from 2.198 to 1.893, and the juices of *Rumex* show a variation between 2.671 and 2.468 from day to night. The filterable iron content of the *Oxalis* plant shows a corresponding range of variation, but in this plant there appears to be no relation between the variation in pH and the varia-

tion in soluble iron content; while *Rumex* shows the definite inverse relation between pH and filterable iron content of plant juices which has been found for many other species. It is to be emphasized that although these fluctuations are no doubt significant, they vary over a rather narrow range.

While the juices of *Rumex* at all times show pH values considerably below the lower limit (pH 3.5) of iron precipitation as indicated by PATTEN and MAINS (**24**), this plant nevertheless shows the same correlation between pH and soluble iron content of tissue fluids as do the species investigated by INGALLS and SHIVE (**15**). On the other hand, *Oxalis*, with extremely low pH values of tissue fluids, shows no such correlation, nor does there appear to be any direct relation between light intensity and pH of tissue fluids in this plant, although both pH values and soluble iron content fluctuate quite as much during a 24-hour period as do these values in *Rumex*. The relations of pH values and soluble iron content for *Rumex* are clearly indicated by the graphs of figure 1, which were plotted from the data of table I.

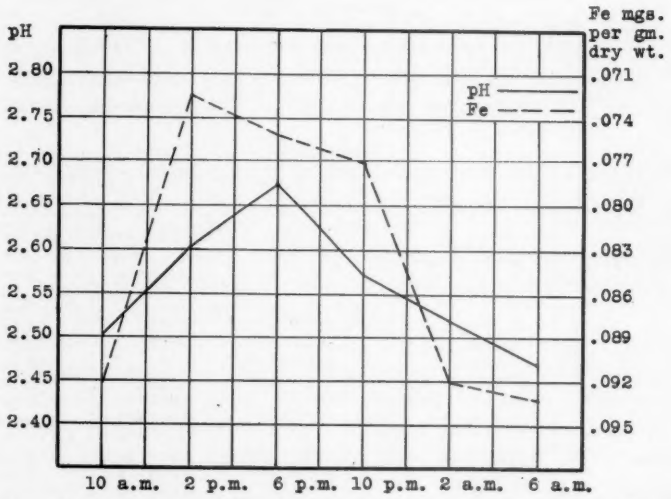

FIG. 1. Graph showing relation of pH to soluble iron content of *Rumex* leaves during 24-hour experimental period.

From the evidence obtained in the experiments with *Rumex*, it may safely be concluded that, although the pH values during a 24-hour period never reach the lowest point (pH 3.5) in the iron precipitation range, as found by PATTEN and MAINS (**24**), the soluble iron content is nevertheless directly correlated with fluctuations in the H-ion concentration.

Soy bean and potato

As may be observed from the data presented in table II and the graphs of figure 2, which were plotted from these data, the soluble iron contents of both the potato and the soy bean plant show, during a 24-hour period,

TABLE II

pH, filterable and total iron content in potato and soy bean plants at different intervals over a 24-hour period

Species	Time of sampling	pH	Iron (mg. per gm. dry weight)	
			Filterable	Total
			mg.	*mg.*
Potato (leaf)	10 A. M.	6.188	0.026	0.612
	2 P. M.	6.576	0.028	0.522
	6 P. M.	6.424	0.029	0.580
	10 P. M.	6.306	0.029	0.498
	2 A. M.	6.272	0.031	0.538
	6 A. M.	6.238	0.034	0.652
Soy bean (whole plant)	10 A. M.	6.002	0.010	0.314
	2 P. M.	6.086	0.010	0.444
	6 P. M.	5.934	0.012	0.484
	10 P. M.	6.103	0.011	0.594
	2 A. M.	6.019	0.016	0.619
	6 A. M.	5.833	0.019	0.682

fluctuations which are closely correlated, in an inverse relation, with the fluctuations in pH values of the tissue fluids. The average pH of the potato is 6.334 and that of the soy bean is 5.996, as indicated in table III.

TABLE III

Average soluble iron, total iron, and pH values for *Oxalis*, *Rumex*, potato and soy bean, over a 24-hour period

Species	pH	Iron	
		Soluble	Total
		mg.	*mg.*
Oxalis (leaf)	2.023	0.058	0.170
Rumex (leaf)	2.563	0.084	0.197
Potato (leaf)	6.334	0.029	0.567
Soy bean (whole plant)	5.996	0.013	0.522

Soluble iron content of the soy bean fluctuates very strikingly following fluctuations in the pH values of tissue fluids. In the juices of these plants, the pH varies from just above the point of complete iron precipitation (as

found by PATTEN and MAINS) to just below this point. The same close relation holds for fluctuations in the potato, with the exception of the first daylight period, although no pH value is as low as the extreme upper limit of iron precipitation. From the foregoing results, it may be concluded that although the pH values of tissue fluids of certain plants may lie consider-

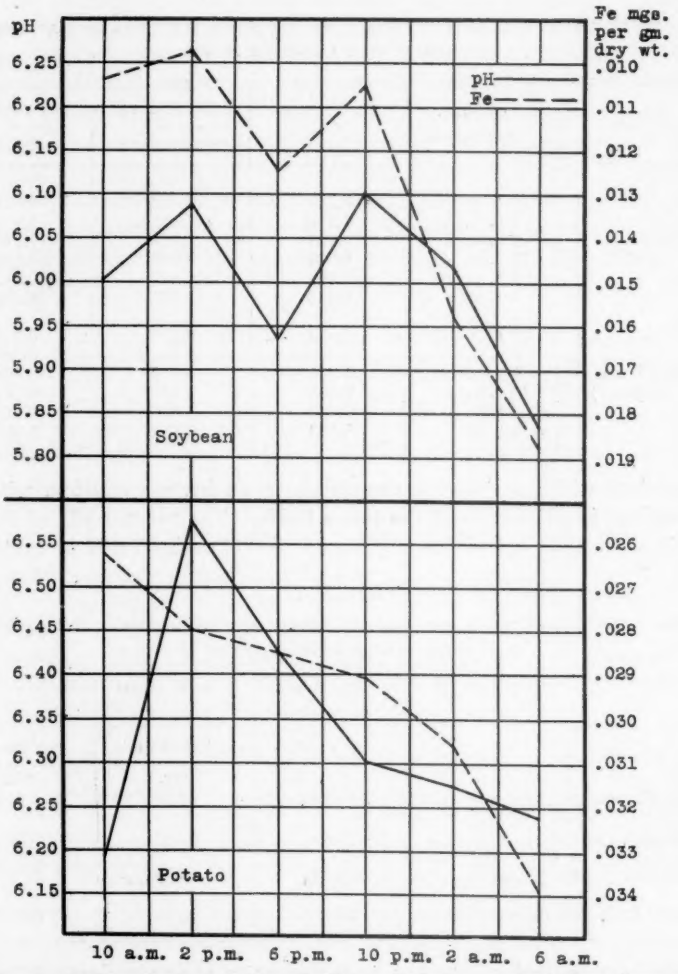

FIG. 2. Graphs showing relation of pH to soluble iron content of soy bean and of potato plants during 24-hour experimental period.

ably above the upper limit of iron precipitation in inorganic solutions, the soluble iron content still rises and falls within a narrow range with the fluctuating pH values resulting from variations in light intensity. This feature of the problem will receive more detailed consideration in a later section, but it might not be out of place here to emphasize several important points.

From the results obtained in this investigation, it is apparent that the range in pH values over which iron precipitation may proceed in simple inorganic solutions, according to the work of PATTEN and MAINS, cannot hold for complex organic systems such as are represented by the tissue fluids in plants. Plants yielding composite tissue fluids with pH values considerably above 6.0 still contain soluble iron, although in relatively low concentrations, yet in sufficient concentration to supply the needs of the plant under favorable conditions. Furthermore, plants yielding composite tissue fluids with pH values considerably below the lower limit of iron precipitation in simple inorganic solutions still contain insoluble iron, or at least iron which does not appear in the filtered plant juices with the methods here employed; although INGALLS and SHIVE (**15**) have shown that in some high-acid plants, practically all of the iron is present in the filterable form, and may be recovered in the filtrate.

It will be seen from tables I, II, and III that plants with juices having high pH values have high total iron and low soluble iron content, while plants with juices having low pH values have low total iron and high soluble iron content. This means that in plants with relatively high acid reaction, most of the iron is in solution; while in plants with relatively low acid reaction, most of the iron is in the insoluble form and accumulates as such. It is more than probable that if, for some reason, all of the iron in the soy bean or potato plant should suddenly become soluble, it would be extremely toxic to the respective plants. Even chlorotic plants, as has already been stated, may have large accumulations of iron present, even in the chlorotic leaves; but this iron may still be unavailable for the processes requiring it in the chlorophyllous cells. Two important questions arise: Where and why do such accumulations of iron occur? Consideration of these questions will be attempted in the following section.

V. H-ion concentration and distribution of iron as determined by microchemical examination of sectional areas

In carrying out this investigation, it was assumed that a microchemical study of sectional areas of plant tissues might bring out some facts with regard to H-ion concentration of tissues, and its relation to distribution of iron, which could not be obtained by making chemical analyses of tissues of different plant organs and determining the pH values of their juices. This proved to be the case.

In these studies, fresh tissue sections were prepared, microchemical tests were made for iron, and pH values of the different tissues over these sectional areas were determined as previously described. Plants were purposely chosen which yielded composite juices having very low and very high pH values. Among the former were *Oxalis repens* and *Rumex acetosella,* and among the latter were such plants as *Zea mays, Solanum tuberosum, Trifolium repens,* and *Glycine max.*

Sections were prepared from plants grown in the field, and also from those grown under controlled experimental conditions in the greenhouse. Great numbers of these tissue sections were prepared and studied, but only a few photographs (made from camera lucida drawings) of representative sectional areas are here presented and discussed. In the diagrams of these sectional areas, iron, as determined by the microchemical tests employed, is indicated in the cells of the different tissues by stippling, and the attempt is thus made to show approximately the relative quantities of iron present in the cells of the various tissues as these were made apparent by microscopical examinations of the sections after chemical treatment. The methods are strictly qualitative, however, and must be so regarded; they do not differentiate between iron in the soluble form and as a precipitate.

The methods employed in the determination of pH values of the different tissues and cells in sectional areas may not be regarded as being particularly accurate, nor has any method yet been devised by means of which great accuracy may be attained in determining pH values of tissues or cells in cross-section. An attempt has been made, however, to determine two values between which it may be regarded that the true pH of the tissues or cells in question is located. In the following consideration of data relating to sectional areas of plant tissues, two such values are always presented.

Oxalis.—The data representing the pH values of the different tissues in a cross-section of the stem of *Oxalis* are presented in table IV. These data indicate that the pH of all the tissues is relatively very low, the values

TABLE IV

Approximate pH values of various tissues of the stem of *Oxalis* studied in cross-section

Tissues	pH values	Tissues	pH values
Cortex	4.2–3.8	Cambium	
Epidermis	2.8–2.6	Xylem	3.0–2.4
Endodermis	2.8–2.2	Pith	4.2–3.8
Phloem	4.4–3.8		

lying between pH 2.8 and 2.2 for endodermis cells, and between pH 4.4 and 3.8 for phloem. The average pH of the composite tissue fluid extracted from the stem of this plant, as previously given, is pH 2.023.

Examination of the diagram of figure 3, showing a cross-section of the stem of *Oxalis,* brings out the fact that the iron in this stem, as determined by microchemical methods, is rather uniformly scattered in all the tissues, and that the iron content of all the tissues is apparently very low, as this is determined by the methods here employed. This uniform distribution and low iron content are characteristic of the conditions throughout the plant. Accumulations of iron were not found anywhere in the tissues of this plant, except for slight accumulations which were sometimes observed in rather large isodiametric cells in the outer cortex.

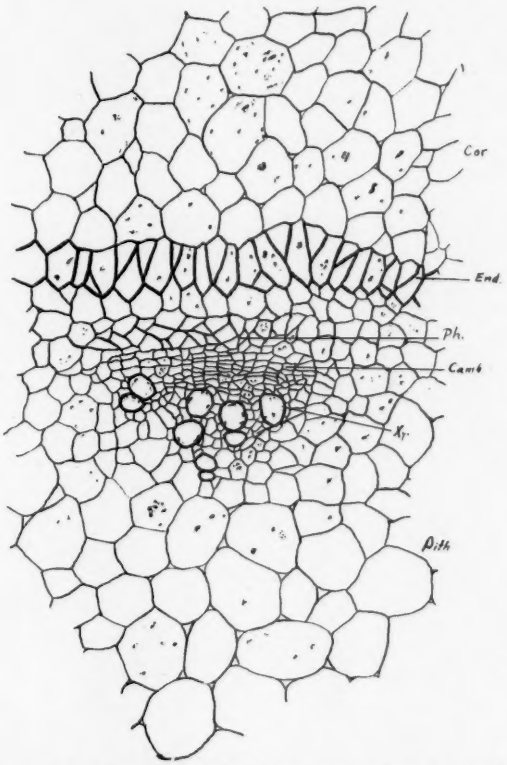

FIG. 3. Section of *Oxalis* stem showing low iron content but uniform distribution of iron in practically all tissues.

While the results obtained by chemical analyses of the plant tissues and of the extracted tissue fluids are in agreement with the results obtained by microchemical and microscopical studies of sectional areas, as here described, the former method does not, by any manner of means, give as detailed or as accurate a picture of the conditions with respect to iron and pH relations as does the latter method.

RUMEX.—As will be observed from the data of table V, the pH values of the tissues across a sectional area of *Rumex* petiole are rather low, ranging from 4.4 to 4.0 for phloem to 2.8–2.4 for sclerenchyma cells. These

TABLE V

APPROXIMATE PH VALUES OF VARIOUS TISSUES OF *Rumex* IN CROSS-SECTION OF LEAF PETIOLE

TISSUES	PH VALUES	TISSUES	PH VALUES
Cortex	4.0–3.6	Xylem	3.0–2.6
Phloem	4.4–4.0	Pith	3.8–3.4
Sclerenchyma	2.8–2.4		

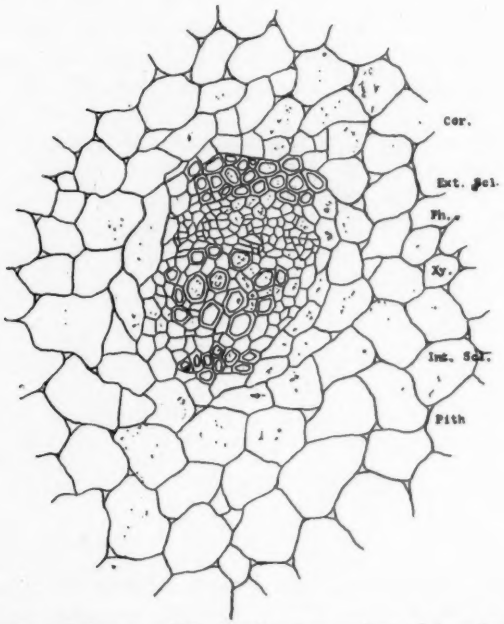

FIG. 4. Section of *Rumex* petiole showing low content of iron but somewhat uniform distribution in practically all tissues.

data are typical of those for sections taken from any part of the plant. The diagram of figure 4, like that of figure 3 for *Oxalis*, shows no accumulation of iron in any of the tissues, but indicates a fairly uniform distribution of iron in relatively small quantities throughout the cross-section.

It is thus apparent that in plants in which all the tissues show relatively low pH values, iron does not accumulate in any one tissue to a greater extent than it does in another. Furthermore, chemical analyses of the filtered tissue fluids of such plants indicate that most of the iron is in the soluble form. It is therefore to be concluded that iron migrates freely in these plants, and that there is no serious obstruction by any of their tissues to its movement from channels of translocation to the chlorophyllous cells of the leaves. Plants of this type have shown no tendency to become chlorotic from lack of iron when grown in artificial culture media containing mere traces of iron, even under experimental conditions definitely unfavorable for growth. These plants present no difficult iron problems, such as are frequently encountered in attempting to grow in artificial growth media plants in which the pH values of the tissues lie close to those representing the upper limit in the range for iron precipitation.

CORN.—A thorough study was made of sectional areas of the corn plant, for the reason that, when growing this plant in culture solutions, some difficulty was always experienced in preventing chlorosis of the leaves caused by the apparent absence of sufficient iron in the leaf mesophyll. The pH values of the composite tissue fluids extracted are relatively high. Table VI, however, shows the xylem and the cells of the bundle sheath to have relatively low pH values; while the phloem, cortex, and leaf mesophyll have high pH values.

TABLE VI

APPROXIMATE pH VALUES OF VARIOUS TISSUES OF STEM AND LEAF OF CORN PLANT STUDIED IN CROSS-SECTION

TISSUES	pH	TISSUES	pH
Xylem (parenchyma)	4.8–4.6	Phloem	6.2–5.8
Xylem (vessels)	4.4–4.0	Cortex	6.0–5.6
Bundle sheath	4.6–4.4	Leaf mesophyll	6.0–5.6
Sclerenchyma	4.8–4.6		

In direct contrast to the conditions found in *Oxalis* and *Rumex*, the corn plant shows very heavy accumulations of iron, primarily in the bundle sheath and in cortical cells immediately surrounding it. These conditions are shown in the diagram of figure 5, which is typical of many sectional

areas prepared from stems and leaves. It is significant that in both the stem and leaf of corn, small amounts of iron were always found in the vessels. This was usually observed in all the plants studied. No iron was found in cortical cells of the stem, nor in mesophyllous cells of the leaf slightly removed from the bundle region. No trace of iron was found in the phloem of the stem or leaf. Apparently iron penetrates the phloem of the leaf and stem with difficulty or not at all. The pH of this tissue is the highest of any found in corn.

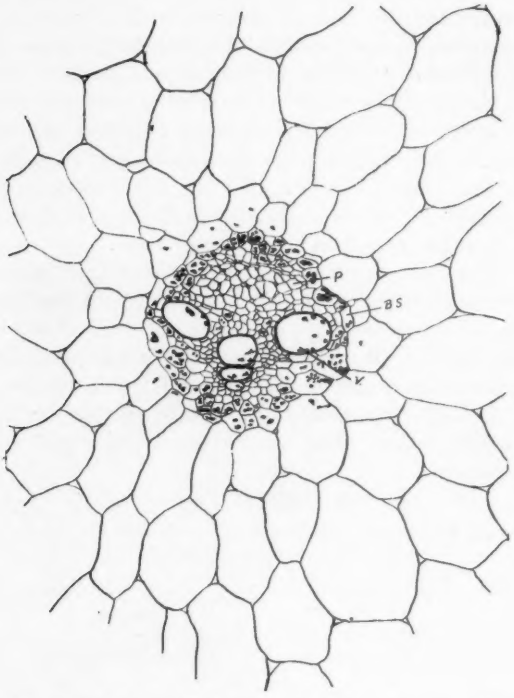

FIG. 5. Section of corn stem showing very heavy iron accumulations in the tissues of the bundle sheath.

It is remarkable to note that in a chlorotic corn leaf, chlorophyll was present only in the mesophyllous cells surrounding the bundles and those near the strip of sclerenchymatous tissue near the upper surface opposite each large vascular bundle. In the leaf blade, beginning a short distance from the midrib, the bundle and this strip of mechanical tissue approach each other rather closely, so that the spongy tissue between the two is only

two or three cells in thickness. In a chlorotic leaf, these cells, as well as those near the bundle and sclerenchymatous tissue, possess chlorophyll, while those between the bundles are devoid of it. Such a condition is externally apparent to the naked eye. The course of the veins is marked by dark green lines, while the space between them is yellow or greenish yellow, depending upon the degree of chlorosis.

A study of sectional areas of chlorotic corn leaves and of green leaves brings out the fact that chlorotic leaves contain as much or more iron than do green leaves, in so far as this can be detected by microchemical methods. This is indicated also by chemical analyses of the tissues in question. In the chlorotic leaves, however, the iron is confined to the bundle regions; and from these regions it penetrates, if at all, only to the cells lying in close proximity to them or to sclerenchyma tissue. This is undoubtedly due, in large measure, to the steep pH gradient existing between the cells of the bundle sheath and those immediately to the exterior.

The root tissues of corn, as a whole, are somewhat more acid than are the corresponding stem tissues. In the roots of corn, iron in very small quantities was found somewhat well distributed, except that small accumulations were sometimes observed in the cells between the endodermis and the xylem, and in the phloem. In the prop roots, larger accumulations were observed in the phloem.

From the foregoing considerations, it is evident that in the corn plant certain tissues may offer considerable resistance to the passage of iron from the channels of translocation to the chlorophyllous cells, and such obstructions may become rather serious under slightly unfavorable growth conditions. In this plant, the morphological structure is such that the channels of iron translocation are completely surrounded by tissues with very high pH values, through which iron migrates with difficulty.

TABLE VII

APPROXIMATE pH VALUES OF VARIOUS TISSUES OF LEAF PETIOLE OF CLOVER AND SOY BEAN STEM STUDIED IN CROSS-SECTION

PLANT	TISSUES	pH	TISSUES	pH
Clover	Xylem (vessels)	4.8–4.6	Phloem	7.2–6.8
	Xylem (parenchyma)	5.6–5.4	Cortex	6.8–6.4
	Sclerenchyma	4.8–4.6	Pith	6.8–6.4
Soy bean	Xylem (vessels)	5.0–4.6	Phloem	7.4–6.8
	Xylem (parenchyma)	6.6–6.2	Cortex	7.0–6.6
	Sclerenchyma	5.0–4.6	Pith	7.2–6.6

CLOVER AND SOY BEAN.—The pH data for clover and soy bean are given in table VII. It will be observed that the tissues of these two legumes, except the xylem and sclerenchyma, show relatively high pH values. In these two plants there is always a steep pH gradient between xylem and phloem, between sclerenchyma and phloem, and between sclerenchyma and cortex; so that from the xylem outward there are regions of relatively low pH which alternate with regions of high pH. The diagrams of figures 6 and 7, which represent respectively cross-sectional areas of petiole of clover and stem of soy bean, show that while iron occurs generally in the xylem tissues, it accumulates to a marked degree in the border regions between xylem and phloem and between sclerenchyma and phloem. This is particu-

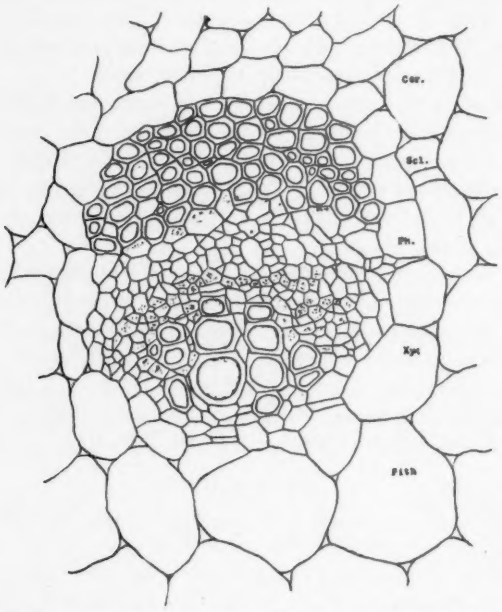

FIG. 6. Section of clover petiole showing iron accumulation in the phloem.

larly true of the soy bean, whose tissues show somewhat higher pH values than do the corresponding tissues of clover. Much of this accumulated iron is without doubt in the precipitated form, since chemical analyses show very high total iron for these plants and exceedingly low total iron for the filtrates of the extracted tissue fluids. Small amounts of iron were generally found also in the cortical tissues of these two species, just outside of low pH sclerenchyma. While iron was not found in this mechanical tissue,

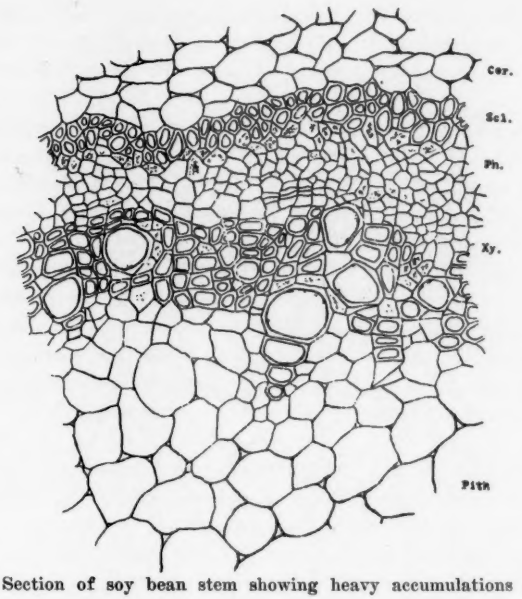

FIG. 7. Section of soy bean stem showing heavy accumulations of iron in the phloem.

except an occasional trace, it appears to have some influence in the translocation of iron into bordering tissues having much higher pH values.

POTATO.—As indicated in table VIII, the pH values of the tissues of the potato plant are very high, ranging from pH 7.6–6.6 for phloem to pH 5.0–4.6 for xylem. There is thus a steep pH gradient between xylem and

TABLE VIII

APPROXIMATE PH VALUES OF VARIOUS TISSUES OF STEM AND PETIOLE OF POTATO PLANT STUDIED IN CROSS-SECTION

TISSUES	PH	TISSUES	PH
Xylem (parenchyma)	6.6–6.2	Cortex	6.8–6.4
Xylem (vessels)	5.0–4.6	Pith	7.0–6.4
Phloem	7.6–6.8		

phloem, with heavy accumulations of iron in the phloem, as indicated in the diagram of figure 8, representing a cross-sectional area of a leaf petiole. Small quantities of iron are also found in the vessels and scattered throughout the cells of the xylem parenchyma.

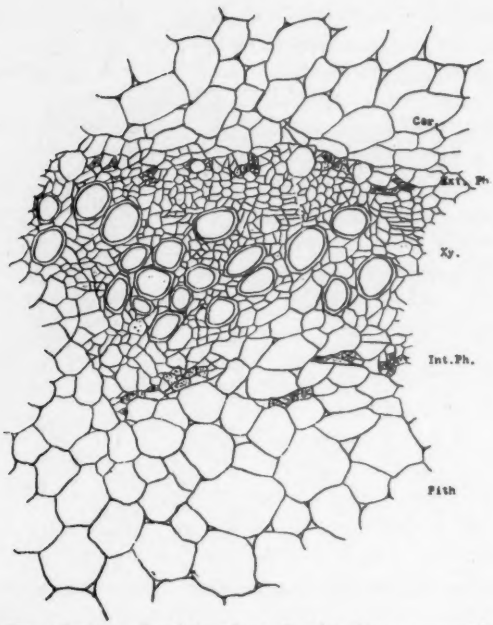

FIG. 8. Section of stem of potato plant showing heavy accumulations of iron in the external and internal phloem.

As has previously been shown, the total iron content of this plant is very high, while the iron content in the filtrate of its extracted juices is very low. It appears, therefore, that the iron accumulation in the internal and external phloem largely represents precipitated or non-functioning iron.

The potato plant, in marked contrast to the corn plant, may be grown in artificial culture media without showing such a strong tendency toward becoming chlorotic as is exhibited by the corn plant in the same medium, although the tissues of the former show considerably higher pH values than do the corresponding tissues of the latter, and in spite of the fact that the tissue fluids of the corn contain a much higher filterable iron content per gram of dry tissue than do those of the potato. It is apparent, therefore, that there is an inherent difference between these two plants with respect to the economy of iron utilization and its transfer from channels of translocation to chlorophyllous cells, for which at the present time no adequate explanation can be offered.

Discussion

A study of the tissues of different plants, and the determination of their approximate pH values, bring out the fact that, in all the plants studied,

the xylem tissues always showed the lowest pH values and phloem the highest. Sclerenchymatous tissues, whenever these occurred, showed pH values which were about the same or slightly higher than those of the xylem.

In the study of iron distribution in the plants, as determined by the microchemical methods here employed, it is significant that small amounts of iron were always present in the vessels and in the xylem parenchyma, but in no instance did pronounced iron accumulation occur in these tissues. On the other hand, marked accumulations of iron, whenever these were present, usually occurred in high pH tissues, such as phloem where this bordered on the xylem or other low pH tissue. As already explained, much or all of the iron in these heavy accumulations may be regarded as being in the precipitated form, not mobile, and therefore cannot function in the metabolic processes of the plant. It is logical to conclude also that the xylem furnishes the tissues in which the upward translocation of iron occurs.

The root tissues of the plants here studied, as a whole, appeared somewhat more acid than did the corresponding tissues of the stem. From the standpoint of pH values, plants such as corn should experience no great difficulty in absorbing iron from the surrounding medium, provided that an available supply is present, since epidermis and subepidermal tissues of the roots of all the corn plants studied were relatively quite acid, and no cortical tissue of roots was found which showed pH values at or above the extreme upper limit (pH 6.0) in the range of iron precipitation as determined by PATTEN and MAINS.

The only tissues in which iron was found to accumulate in the corn roots were the cells between the endodermis and xylem and in the phloem, and these accumulations were found only occasionally and were not particularly pronounced. In all the species investigated, iron was found in small quantities in the young roots, but no accumulations were observed in these organs, the iron being rather uniformly distributed throughout the tissues.

Although iron is directly or indirectly necessary for chlorophyll production, it is apparent that if the chlorophyllous cells are comprised in a tissue with a pH reaction near or above the upper limit in the range of iron precipitation, the iron may be prevented from entering or penetrating such tissue because of precipitation, as a result of which a chlorotic condition may occur. As already pointed out, however, the range in pH values over which iron precipitation occurs in some plants is wider than that indicated by PATTEN and MAINS for inorganic systems. Hence in certain plants it is possible that iron in small quantities may penetrate tissues having pH values considerably above the upper limit (6.0) of this range, while in others it may not be possible for iron to penetrate such tissues. The former

type of plant may show no tendency toward chlorosis, when subjected to a given set of conditions under which the latter may suffer severely from chlorosis owing to lack of iron in chlorophyllous cells. Two species which showed this pronounced contrast under like experimental conditions are corn and potato, the former showing a marked tendency toward chlorosis from lack of iron in chlorophyllous tissues under certain experimental conditions, while the latter showed no such tendencies under the same conditions, in spite of the fact that the potato plant contains a much lower percentage of soluble (filterable) iron than does the corn plant. The plants of both these species contain a large excess of total iron, most of which is in the insoluble (non-filterable) form.

Many agricultural plants with tissues having high pH values show these same inherent differences, for which, as has previously been pointed out, no adequate explanation can be offered at the present time on the basis of experimental evidence. It may be suggested, however, that certain organic iron compounds are able to resist the precipitating action of alkaline media, and that iron in the presence of certain organic compounds is retained in solution form in alkaline media. MARSH and SHIVE (**19**) have shown that, of the four iron compounds (ferric glycerophosphate, soluble ferric phosphate, ferric tartrate, and ferrous sulphate) in solution in a culture medium at pH 6.2, no ferrous sulphate and no soluble ferric phosphate remained in solution at the end of a given experimental period; while 40 per cent. of the iron present in the same medium as ferric tartrate and 20 per cent. as ferric glycerophosphate remained in solution at the end of the period under the same experimental conditions.

REED and HAAS (**25**), in a series of qualitative tests, found that tartrates and citrates were effective in holding iron in solution in an alkaline medium. Citrates were somewhat more effective in this respect than tartrates. By testing for iron with potassium thiocyanate, they found abundant quantities in solution in a medium at pH 7.6, with sodium citrate present. FISCHER (**7**) obtained similar results using glycerol in an alkaline medium. DAKIN (**6**) mentions that this also holds true for hydroxyaspartic acid, although he gives no experimental evidence. GILE and CARRERO (**8, 9, 10**) found that, of a number of iron sources, only ferric tartrate furnished sufficient iron for rice grown in alkaline solutions. When judged by the growth of rice plants, ferrous sulphate, ferric citrate, and ferric tartrate afforded sufficient iron, when used in proper quantities in acid and neutral solutions. Many other workers have found similar results with certain organic compounds.

SMYTHE and SCHMIDT (**28**) state that those substances which possess a certain particular grouping within the molecule will hold iron as an undissociated compound. Such a combination in most cases is rather resistant

to the action of alkaline solutions. They found the following classes of substances to possess the necessary grouping: hydroxymono-carboxylic acids (lactic, gluconic); dicarboxylic acids (oxalic, malonic); hydroxydicarboxylic and hydroxytricarboxylic acids (tartaric, citric); amino acids which are also hydroxy- or dicarboxylic acids (aspartic acid, serine); certain inorganic acids (phosphoric, arsenic); certain phosphorus-containing compounds (nucleic acid, glycerophosphoric acid); and certain proteins (casein, gelatin). They suggest an explanation, based on the residual charge of ions, for the manner in which the iron may be united.

It appears logical to assume that, if such compounds as those just mentioned serve to hold iron in solution in an external alkaline medium, they should serve the same purpose inside the plant, provided that they are present there. Many such compounds are known to be present in some plants, and certain ones may be entirely lacking in others. It may then be suggested as a possibility that some plants with high pH tissues may show little tendency toward chlorosis, even under somewhat unfavorable growth conditions, because they may contain certain organic solvents, in the presence of which iron in the mobile form resists the precipitating influence of high pH reactions. On the other hand, certain plants with high pH tissues may show a strong tendency toward chlorosis, because they may not contain the particular organic solvents which hold iron in solution in the tissues against the precipitating influence of high pH reactions.

It seems probable that the precipitated iron which is present in certain tissues of high pH plants may serve as a reserve iron supply, in that certain of these organic solvents may move across such tissues containing the precipitated iron, and may become effective factors in translocating some of it to tissues more remote.

Summary

1. Plants which yield composite tissue fluids having high pH values in general show very low soluble (filterable) iron content and very high total iron.

2. Plants which yield composite tissue fluids having low pH values in general show relatively high soluble iron and low total iron. In some cases nearly all of the iron in the plant samples appears in the filtered extracts.

3. The range of pH values over which iron appears to precipitate in plants of different species is wider than the corresponding range for inorganic systems.

4. Plants whose extracted tissue fluids show very high pH values, and those with tissue fluids having very low pH values, still show slight fluctuations in soluble iron content over a day and night period, which corresponds in the inverse relation to fluctuations in pH values of the tissue fluids due to variation in light intensity over the same period.

5. The highest pH values of specific tissues for each species investigated occurred in the phloem, with cortex only slightly lower; and the lowest pH in the xylem. Steep pH gradients always occurred between xylem and phloem.

6. Iron accumulations usually occurred in high pH tissues lying adjacent to relatively low pH tissues with a steep pH gradient between. It is concluded from chemical analyses that iron in these accumulations is in a precipitated form and not available for plant processes.

7. No iron accumulations were found in plants with low pH tissues throughout. In these plants the iron content is low and uniformly distributed in practically all of the tissues.

8. The xylem furnishes the main channels of iron translocation from the roots to the leaves.

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HYDROGEN-ION CONCENTRATION AND IRON CONTENT OF TRACHEAL SAP FROM GREEN AND CHLOROTIC PEAR TREES

J. OSERKOWSKY

(WITH TWO FIGURES)

Introduction

The occurrence of chlorosis on muck soils to which lime was added was found by LOEHWING (**4**) to be correlated with low acidity of the sap of plants growing on these soils, as compared with the H^+ concentration of the sap from similar plants grown on unlimed soils. It was held that on the limed soils the iron failed to reach the leaves in sufficient amounts, because the iron was precipitated in the roots and the stems, on account of the low acidity of the sap in these parts. MARSH and SHIVE (**5**) observed that the iron content of stems of chlorotic soy beans was higher than that of stems from green plants, while the iron content of the chlorotic leaves was lower than that of green leaves.

The work just referred to was conducted with annual plants. It has been observed, however, that chlorotic leaves from fruit trees may contain as much or more iron than green leaves (**7**). Further, MILAD (**6**) and WALLACE (**8**) did not find a significant difference between the iron content of bark or wood of stems and roots from chlorotic and green trees. These findings indicate that chlorosis cannot be explained solely on the assumption that iron fails to reach the leaves in sufficient amount for normal development of chlorophyll. If iron moves to the leaves in the tracheal sap, then the question arises, is the H^+ concentration of the tracheal sap sufficiently high to prevent the absorbed iron from precipitating in the stem before reaching the leaves? The present work was undertaken in an attempt to answer this question.

Material and methods

Three series of branches of Hardy pear trees were collected from an orchard situated in the Santa Clara Valley in California. The soil in this locality is rich in lime, as a consequence of which "lime-induced chlorosis" is common. Practically all the trees in this orchard suffered from chlorosis, which was found to be curable only by administration of iron salts to the trees. One series comprised branches from normally green trees; another series comprised branches from chlorotic trees which were treated with iron citrate, as a consequence of which they developed deep green leaves; and a third series comprised chlorotic branches. A fourth series of samples

was taken from another orchard situated also in the Santa Clara Valley, but in which all the trees were healthy and green.

The samples were collected at different times of the day, but all branches collected at any date were taken at the same time of the day, in order to eliminate the effect of possible diurnal differences in pH. As soon as the branches were severed from the trees, the leaves and twigs were removed in order to prevent loss of water by transpiration. The branches were then removed to cold storage (36° F.) over night, after which the tracheal sap was extracted according to the method of BENNETT, ANDERSSEN, and MILAD (3). The pH of the sap obtained from each branch was determined separately by the quinhydrone electrode. The results obtained with this electrode were in fair agreement with those secured by the colorimetric method. Each of the pH values in table II represents an average of four to six branches.

While it is possible that the pH of the tracheal sap underwent some changes during storage, experience in this laboratory had shown that the magnitude of these changes hardly exceeds a few tenths of a pH unit. Such changes are considered insignificant, since they are of the same order of magnitude as differences in pH between branches of the same series collected at the same time. The variation in pH of the tracheal sap of such samples can be gauged from table I, in which are presented the pH

TABLE I

H^+ CONCENTRATION OF EXTRACTED TRACHEAL SAP OF CHLOROTIC PEAR BRANCHES

	No. of branch												
	1	2	3	4	5	6	7	8	9	10	11	12	13	Average
pH	5.52	5.49	5.45	5.34	5.40	5.14	5.45	5.47	5.47	5.60	5.52	5.55	5.72	5.48

values of sap obtained from thirteen branches collected on April 1 at the same hour of the day.

The determination of iron in the tracheal sap was carried out as follows. Aliquots of the sap were evaporated in porcelain crucibles, ashed, dissolved in aqua regia, evaporated to dryness, dissolved in 0.5 N NHO_3, and made up to a definite volume. To an aliquot of this solution KCNS, and ethyl-acetate or amyl-alcohol, were added, the mixture shaken, allowed to stand for a few minutes, and the iron in the amyl-alcohol or ethyl-acetate phase determined colorimetrically. Determinations of iron in blanks were made, and the results given in table II corrected accordingly.

Results and discussion

Figure 1 shows that the H^+ concentration in the tracheal sap is higher at the beginning of the season than later. The period of high acidity lasts in green trees for about six to eight weeks, while in chlorotic trees it is

TABLE II

CONCENTRATION OF H' AND IRON IN THE TRACHEAL SAP OF GREEN AND CHLOROTIC BRANCHES FROM HARDY PEAR TREES

DATE OF COLLECTING SAMPLES	pH OF TRACHEAL SAP				IRON IN TRACHEAL SAP (MG. Fe PER LITER)			
	SERIES OF SAMPLES							
	GREEN BRANCHES FROM HEALTHY TREES IN CHLOROTIC ORCHARD	GREEN BRANCHES FROM TREES TREATED WITH IRON	CHLOROTIC BRANCHES	GREEN BRANCHES FROM HEALTHY ORCHARD	GREEN BRANCHES FROM HEALTHY TREES IN CHLOROTIC ORCHARD	GREEN BRANCHES FROM TREES TREATED WITH IRON	CHLOROTIC BRANCHES	GREEN BRANCHES FROM HEALTHY ORCHARD
March 12	.....	.....	6.65	.....	.....	.....	.....	.....
" 16	.....	.....	6.92	.....	.....	.....	−0.14	.....
" 31	.....	5.59	5.69	.....	.....	10.0	.....	0.22
April 1	.....	.....	.....	5.76	.....	.....	.....	0.36
" 5	5.64	.....	.....	.....	0.29	.....	0.087	.....
" 6	.....	.....	.....	5.79	.....	.....	.....	.....
" 21	.....	.....	.....	5.76	.....	.....	.....	0.61
" 26	5.72	.....	5.40	.....	0.53	.....	0.49	.....
May 1	.....	.....	.....	.....	0.17	0.21	0.10	0.19
" 28	5.69	5.72	6.27	5.94	.....	.....	.....	.....
June 15	5.84	5.94	6.19	5.85	.....	0.34	0.17	0.20
July 6	.....	.....	.....	6.50	.....	.....	.....	.....
" 9	6.18	6.18	6.17	.....	0.047	.....	0.11	.....
" 11	.....	.....	.....	6.29	.....	.....	.....	.....
Aug. 22	6.69	6.87	6.68	6.90	0.099	0.36	0.13	0.17
Sept. 19	6.72	.....	6.79	6.89	0.075	0.10	0.037	0.24
Oct. 21	.....	.....	.....	.....	0.33	.....	.....	.....
" 30	7.12	7.04	7.00	7.20	.....	.....	0.015	0.075
Dec. 18	.....	.....	.....	.....	0.22	.....	.....	.....
" 28	.....	.....	.....	.....	.....	0.15	.....	0.13
Feb. 7	.....	.....	.....	.....	0.097	.....	0.16	0.12

shorter. The same difference between chlorotic and green trees was observed also in Bartlett pear trees grown in the Santa Clara Valley. This difference in the H^+ concentration is correlated, presumably, with growth activity of the trees.

Except for the difference in the duration of the period of high acidity, no significant difference exists between the pH of the tracheal sap of green and chlorotic trees. The H^+ concentration of the sap from chlorotic branches may be equal to or greater than that of green branches collected at the same date and at the same hour of the day. The concentration of iron in the tracheal sap of branches from the healthy orchard is higher practically throughout the season than that of the chlorotic branches. The "healthy orchard" referred to was situated in a lime soil, and, although the trees in this orchard could not be classed as chlorotic, there were indications in the form of slight traces of chlorosis that they were on the verge of becoming so. Additional data, not reported in this paper, indicate that the iron content of the tracheal sap of branches from Bartlett pear trees grown in non-calcareous soil is, on the whole, higher than that of branches from trees grown in lime soils.

It should be borne in mind that the extracted tracheal sap is in equilibrium with the atmospheric air; while in the plant the tracheal sap is in contact with the gases of the cellular and intercellular spaces, which are in all probability much richer in CO_2 than in air. Since the tracheal sap possesses a weak buffer capacity (**1**), it is very likely that *in situ* its H^+ concentration may be materially affected by the CO_2 concentration of the internal gas. The pH of the extracted sap may not, therefore, represent

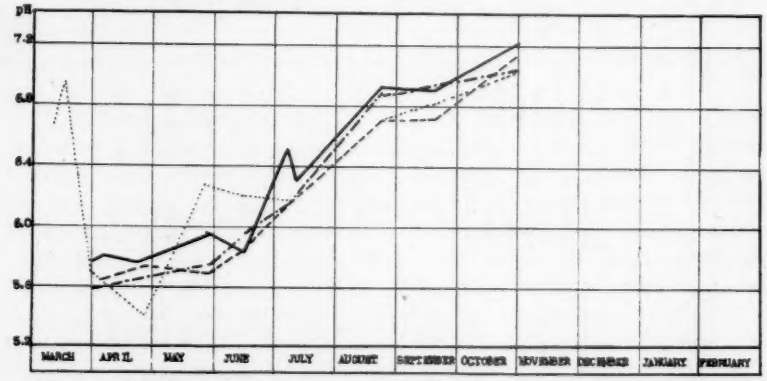

FIG. 1. Seasonal changes of H^+ concentration in tracheal sap of branches from Hardy pear trees. Legend: ———— green branches from healthy orchard; -.-.-. green branches from trees treated with iron; ..... chlorotic branches; – – – – green branches from healthy trees in chlorotic orchard.

the true H^+ concentration of the tracheal sap in the plant. Since, however, it seems that most of the CO_2 present in the sap escapes from it after its extraction, it is believed that the amount of iron in the extracted sap is substantially the same as that of the tracheal sap *in situ*.

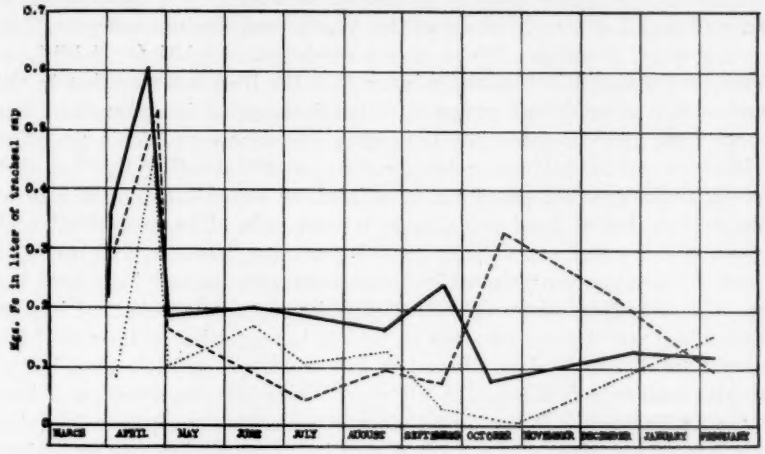

FIG. 2. Seasonal changes in concentration of iron in tracheal sap from branches of Hardy pear trees. Legend: ———— green branches from healthy orchard; ..... chlorotic branches; - - - - - green branches from healthy trees in chlorotic orchard.

The data relating to the concentration of iron in the tracheal sap are presented in table II and figure 2, from which it is seen that, at the beginning of the growth season, the concentration of iron increases very rapidly and then falls abruptly. This rise and fall takes place during the swelling of the buds and blossoming; it lasts three to four weeks. It yet remains to determine what portion of the iron in the tracheal sap during this period is derived from the stored reserves in the plants.

The concentration of iron in the sap fluctuates during the rest of the season, but remains on the whole at a lower level. In the sap from trees which were treated with iron[1] during the winter, before growth started, the concentration of iron was higher during the first part of the season than it was in sap from untreated trees, which was to be expected.

The fact that concentration of iron in the tracheal sap falls shortly after the beginning of the growth season is no indication that the rate at which iron is absorbed by the tree decreases. Indeed the rate of accumulation of iron in leaves is practically constant throughout the growing season.

[1] The iron was administered to the trees in the form of iron citrate powder introduced at the bottom of the trunk. For details concerning the method of treatment, see BENNETT (2).

The decrease in iron concentration in the tracheal sap may be due, at least partly, to the increased rate of transpiration of the tree as the area and number of leaves increase with the advance of the season.

It has just been shown that both the H^+ and iron concentrations of the tracheal sap are higher early in the season than later. On examination of figures 1 and 2, it may be observed that the H^+ and the iron concentrations for any series of samples fail to show a consistent correlation. Additional data, not presented in this paper, show that the iron concentration in the tracheal sap of pear trees grown in non-calcareous soil is higher than that of sap from green or chlorotic trees grown in lime soils. This condition holds true throughout the growing season, notwithstanding the fact that the pH of the tracheal sap of the trees from the non-calcareous soil may be higher than that of trees grown in calcareous soils. The concentration of iron in the tracheal sap of pear trees is very low, however, and may fall short of the saturation point of iron consistent with the existing pH of the sap. In view of this fact, the extent to which the concentration of iron in the tracheal sap of pear trees may be subject to regulation by the pH of the sap yet remains to be determined. It does not seem probable that, in the samples dealt with in this paper, pH was limiting the concentration of iron in the tracheal sap. It is plausible, however, that in those plants in which iron is more abundant in the tracheal sap, its concentration may be limited by the H^+ concentration of the sap.

Conclusions

1. The concentrations of H^+ and iron in the tracheal sap of pear trees reach a high level at the beginning of the growing season; but the period at which the concentration of iron is high is shorter than the period of low pH of the tracheal sap.

2. No significant difference exists between the H^+ and the iron concentrations in the extracted tracheal sap of green and chlorotic branches from the same orchard; the concentration of iron in the tracheal sap from chlorotic trees may be higher than that from green trees.

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USE OF EXPRESSED SAP IN DETERMINING THE COMPOSITION OF CORN TISSUE[1]

J. D. Sayre and V. H. Morris

(WITH FIVE FIGURES)

The composition of sap expressed from plant tissues has been much studied during recent years in various physiological investigations. Most of the investigators, however, have not attempted to relate the composition of the expressed sap to that of the original tissue. In a recent paper on the use of expressed sap in physiological studies of corn (**6**), it was shown that the sugar content of corn tissues could be estimated from the analysis of the expressed sap with a fair degree of accuracy. Certain refinements in technique and calculation have been applied to this method of determining the sugar content of corn tissues with more satisfactory results. The determination of the composition of corn tissues with respect to other constituents by the analysis of expressed sap also has been investigated. The results of these studies are presented in this paper.

The apparatus used for expressing the sap from corn tissues and the details of the general technique were similar in all essentials to those described previously (**6**).

Determining the composition of the tissue from analysis of the expressed sap

Moisture content of the tissue as determined by this method represents the sum of the moisture in the press cake plus the moisture in the expressed sap (**7**). The moisture in the press cake was determined by drying in an air oven, and that of the expressed sap by the refractometer method of Gortner and Hoffman (**3**). The total solids content of sap from leaf tissue determined by the refractometer was consistently higher than that determined by vacuum oven drying. The relation between the methods, based on determinations made at regular intervals during the season, is shown in figure 1. This regression may be used to correct the determinations by the refractometer method in sap from leaf tissue of corn. No correction in sap from stem tissue is necessary.

It was shown in the previous paper (**6**) that the total sugar content of corn tissue can be calculated from an analysis of expressed sap with a fair degree of accuracy. In this calculation the assumption is made that the

[1] Based on investigations cooperative between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Department of Agronomy, Ohio Agricultural Experiment Station.

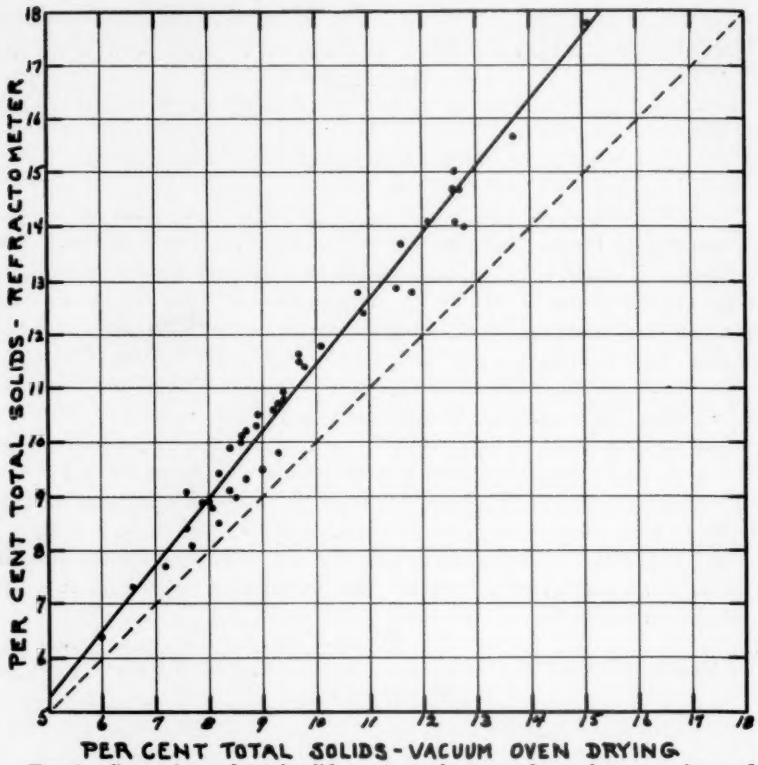

FIG. 1. Comparison of total solids content of expressed sap from corn leaves determined by the refractometer method and by vacuum oven drying.

composition of the sap expressed is similar in sugar concentration to the sap remaining in the press cake. In calculating the sugar content of the tissue, no correction was made for the specific gravity or the total solid content of the sap; consequently the results were slightly lower than those obtained by the standard alcoholic extraction method. Additional determinations were made during the summer of 1930, correcting for these facts.

In order to determine the sugar content of corn tissue from an analysis of the expressed sap, it is necessary to know the moisture content of the tissue and the sugar content and moisture content of 100 ml. of the expressed sap. From these data, the percentage of sugar in the tissue is calculated by the following formula:

$$\text{Per cent. of sugar in tissue} = \frac{\text{Grams of sugar in 100 ml. sap}}{\text{Grams of moisture in 100 ml. sap}} \times \text{total moisture content of tissue, grams}$$

A method essentially similar to this has been used by MASON and MASKELL (**4**).

The sugar analyses were made on 50 ml. of sap preserved by boiling in alcohol and the results expressed in grams of sugar in 100 ml. of the sap. The grams of moisture in 100 ml. of sap were obtained by subtracting the corrected total solids, as determined by the refractometer, from the weight of the sap, determined through its specific gravity.

A comparison of the results obtained by the sap expression method and the alcoholic extraction method for determining sugar in corn tissue was made on a series of 24 samples, comprising leaf, stem, and tassel tissue, taken during the period from July 7 to September 8, 1930. Free reducing sugars, sucrose, and total sugars were determined on each sample by both methods. The determinations, entered in columns a and b (table I), under each method, represent single determinations on duplicate aliquots from each sample of tissue, expressed on a green weight basis.

The two methods, sap expression and alcoholic extraction, were compared with regard to: (1) agreement in determining the mean sugar content of all the samples; (2) agreement in determining the sugar content of different kinds of tissue in varying stages of development; and (3) the relative precision of the methods in determining the sugar in duplicate samples. The mean free reducing sugar content of the 24 samples was 2.618 per cent. by the sap expression method and 2.656 per cent. by the alcoholic extraction method; the mean sucrose contents were 1.660 and 1.711 per cent. respectively; and the mean total sugar contents were 4.359 and 4.445 per cent. The significance of the differences between these pairs of mean values was tested, using FISHER'S (**1**) formula, $t = \frac{\bar{x}}{s}\sqrt{n}$, and the probability tables of STUDENT (**8**). The differences were such as might be due to chance, the odds not exceeding 14:1 in any comparison. It may be concluded, therefore, that the mean sugar contents of all the samples, as determined by the sap expression and by the alcoholic extraction methods, are in good agreement.

The product moment coefficients of correlation were 0.960, 0.993, and 0.996 for free reducing sugars, sucrose, and total sugars respectively. These are high enough to show that the methods are equally satisfactory over the entire range included.

The standard errors for the sap expression and alcoholic extraction methods were: free reducing sugars, 0.089 and 0.108 per cent.; sucrose content, 0.072 and 0.110 per cent.; and total sugars, 0.092 and 0.142 per cent. In each case the standard error for the sap expression method was less than that for the alcoholic extraction method. It may be concluded from these results that the sugar content of corn tissue can be determined by the sap

TABLE I

COMPARISON OF SAP EXPRESSION AND ALCOHOLIC EXTRACTION METHODS OF SUGAR DETERMINATION

		Free reducing sugars				Sucrose				Total sugars			
		Expressed sap		Alcoholic extraction		Expressed sap		Alcoholic extraction		Expressed sap		Alcoholic extraction	
		a	b	a	b	a	b	a	b	a	b	b	b
		per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
July 7	Leaves	0.93	0.91	0.91	0.92	0.59	0.59	0.62	0.72	1.56	1.54	1.58	1.70
" 16	Leaves	1.48	1.51	1.47	1.40	0.81	0.86	1.05	1.12	2.33	2.43	2.57	2.57
	Stems	3.83	3.88	3.86	3.93	0.83	0.77	0.85	0.77	4.71	4.69	4.76	4.74
	Tassels	2.55	2.53	2.64	2.98	0.90	0.81	0.76	0.87	3.49	3.38	3.44	3.50
" 18	Leaves	2.39	2.41	2.24	2.24	0.09	0.02	0.00	0.06	2.49	2.43	2.23	2.30
	Leaves	2.09	2.48	2.09	2.04	0.40	0.17	0.26	0.53	2.66	2.67	2.36	2.60
" 22	Leaves	1.58	1.47	1.27	1.27	0.45	0.59	0.95	0.95	2.05	2.11	2.27	2.27
	Lower stems	3.81	3.94	3.63	3.63	0.26	0.37	0.76	0.57	4.19	4.20	4.43	4.23
" 29	Leaves	2.15	2.37	1.82	1.81	0.61	0.78	0.85	0.72	2.80	3.19	2.72	2.57
	Rolled leaves	2.65	2.42	3.00	3.19	0.92	0.93	1.13	1.07	3.62	3.39	4.19	4.32
	Tassels	2.77	2.93	2.74	2.46	0.88	0.99	1.04	0.99	3.71	3.97	3.83	3.50
" 31	Leaves	2.39	2.39	2.86	2.75	0.31	0.28	0.53	0.04	2.72	2.68	3.42	2.79
	Lower stems	4.88	4.92	4.87	4.85	1.16	1.13	1.24	1.15	6.11	6.11	6.18	6.06
Aug. 5	Leaves	1.94	1.97	1.74	1.74	0.89	0.76	0.91	0.95	2.88	2.76	2.70	2.74
	Lower stems	4.25	4.37	4.14	4.14	2.40	2.40	2.36	2.39	6.77	6.90	6.62	6.66
" 11	Leaves	1.90	1.83	2.00	2.04	1.55	1.57	1.16	1.20	3.53	3.48	3.22	3.30
	Lower stems	3.90	3.86	4.04	4.36	4.30	4.30	4.39	4.41	8.42	8.38	8.66	9.00
" 12	Leaves	2.26	2.27	2.87	3.00	1.22	1.14	0.68	0.50	3.55	3.46	3.59	3.53
" 18	Leaves	2.17	2.17	2.10	2.28	0.98	0.95	0.89	1.03	3.20	3.17	3.22	3.18
	Lower stems	3.56	3.44	3.80	3.86	6.33	6.48	6.45	6.47	10.02	10.04	10.54	10.50
" 25	Leaves	1.34	1.41	1.48	1.42	1.21	1.14	1.05	1.07	2.61	2.61	2.58	2.54
	Lower stems	3.59	3.63	4.02	4.10	5.68	5.72	5.55	5.55	9.57	9.64	9.86	9.94
Sept. 8	Leaves	1.92	1.84	1.44	1.34	1.16	1.25	1.56	1.65	3.14	3.16	3.08	3.08
	Lower stems	2.21	2.17	2.38	2.34	5.97	5.80	6.02	6.29	8.46	8.28	8.68	9.00
	Mean	2.618		2.656		1.660		1.711		4.359		4.445	

expression method at least as accurately as by the alcoholic extraction method.

Relation between composition of successive portions of expressed sap and composition of the tissue from analysis of the expressed sap

It was desirable to analyze successive portions of expressed sap to check the uniformity of sugar content and also to determine other substances in the successive portions. The sap expressed from a sample of ground tissue was collected, 10 ml. at a time, as it came from the press cage, and each portion analyzed separately for total solids by the refractometer and by vacuum oven drying, for total sugars, total nitrogen, nitrate nitrogen, total phosphorus, and inorganic phosphorus.

The total solids, as determined by the refractometer and by vacuum oven drying, and the total sugars are shown in figure 2. Total solids decreased in successive portions of the sap, vacuum oven drying giving values

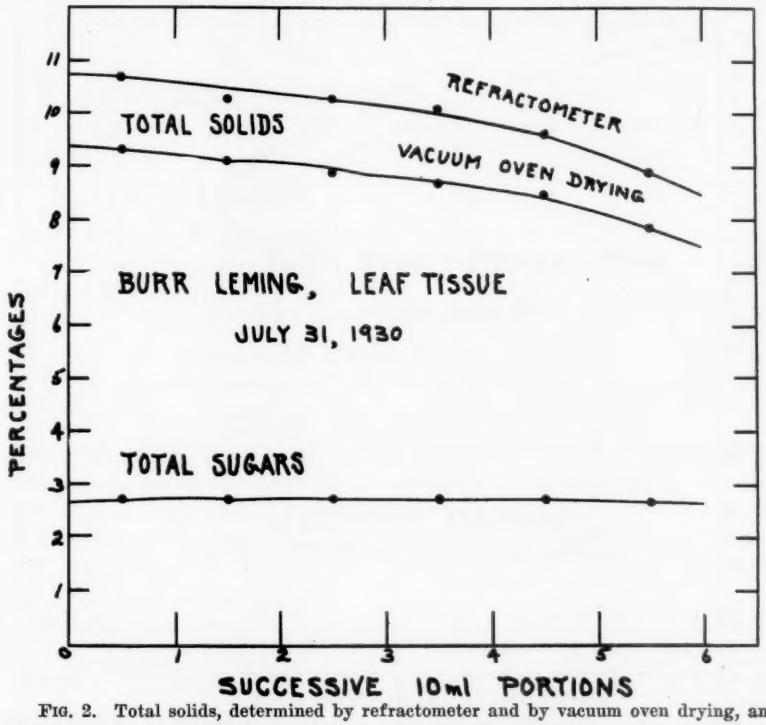

FIG. 2. Total solids, determined by refractometer and by vacuum oven drying, and total sugars in successive portions of sap expressed from leaf tissue of Burr-Leaming corn.

similar to those obtained by the refractometer but uniformly lower. Total sugars were similar in each successive portion of the sap. Total solids in the expressed sap were composed of water-soluble and water-miscible materials from the tissue. The total solids in the first portions represent some material from the broken cells in the outer part of the mass of ground tissue in the press cage. The decrease in these materials in later portions of the expressed sap is probably due to the filtering action of the mass of tissue itself. Sugars, being in true solution in the water of the tissue, are not removed by this filtering action. Since the total sugars are constant in each successive portion of the sap, it is assumed that they are also contained in the water which remains in the press cake in this same proportion.

The total nitrogen, nitrate nitrogen, total phosphorus, and inorganic phosphorus content of the successive portions are shown in figures 3 and 4. Total nitrogen and total phosphorus decreased in each successive portion of the sap; while nitrate nitrogen and inorganic phosphorus were practi-

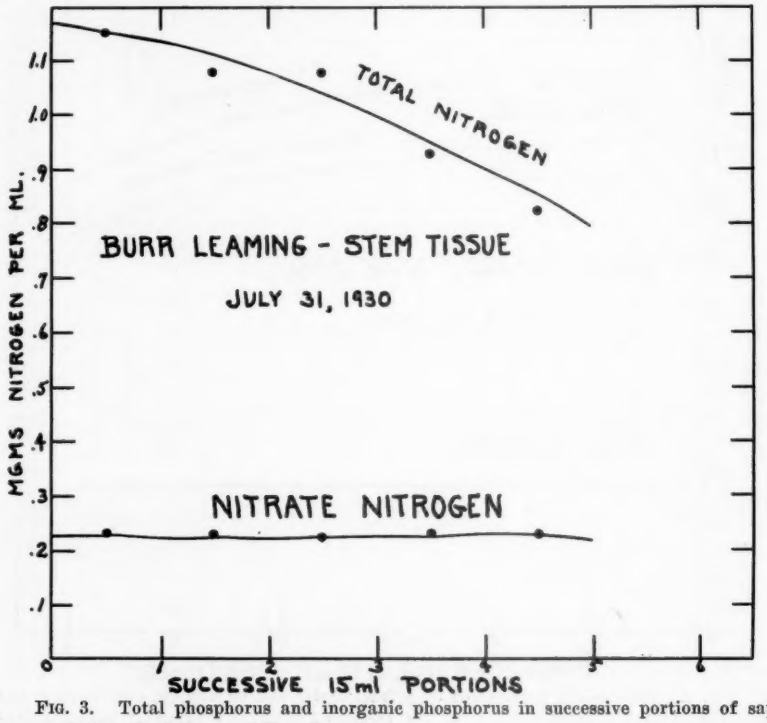

FIG. 3. Total phosphorus and inorganic phosphorus in successive portions of sap expressed from leaf tissue of Burr-Leaming corn.

cally constant in the successive portions. These results, including those obtained on sugars, indicate that when tissue is ground and the sap removed by pressure as outlined, those substances in true solution are constant in the successive portions of the sap. It follows that the composition of the tissue with respect to such substance can be calculated from analysis of the expressed sap.

A comparison of the nitrate nitrogen content of corn tissue as determined by the sap expression and the alcoholic extraction methods has been reported by GERDEL (**2**), who found excellent agreement between the two methods.

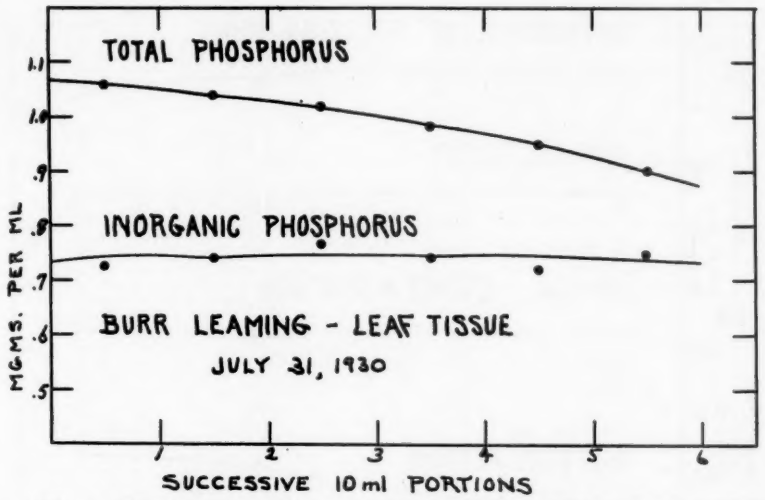

FIG. 4. The nitrogen and nitrate nitrogen in successive portions of sap expressed from stem tissue of Burr-Leaming corn.

The conductivity, freezing point, pH, and buffer action of successive portions of expressed sap were determined. The results (fig. 5) show that there was very little change in the composition of the successive portions in these properties, with the exception of the buffer action. Similar results were reported by NEWTON, BROWN, and MARTIN (**5**), in expressed sap from wheat plants.

Since the freezing point, conductivity, and pH of successive portions of sap are similar, it may be assumed that they are also similar in the sap remaining in the press cake. It is impossible, however, to tell what has happened during the grinding of the tissue (necessary to obtain a satisfactory sample of expressed sap), and thus nothing can be concluded concern-

ing the conductivity, freezing point, or pH of the unaltered tissue. Until more satisfactory methods of measuring these properties in the living tissue are devised, no conclusions can be reached in this connection.

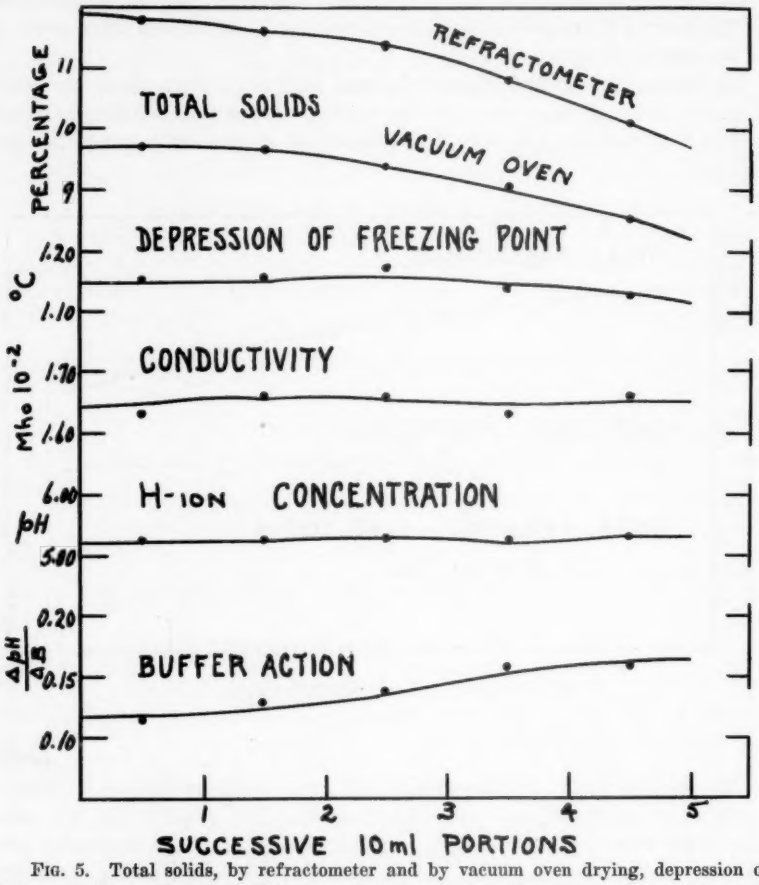

FIG. 5. Total solids, by refractometer and by vacuum oven drying, depression of freezing point, conductivity, H-ion concentration, and buffer action of successive portions of sap expressed from leaf tissue of Burr-Leaming corn.

Effect of varying size of samples, pressure, and preliminary treatment of tissue on determination of composition of the tissue by analysis of expressed sap

In the experiments reported above, a 100-gram sample of ground corn tissue, subjected to a pressure of 5000 pounds per square inch for 5 minutes,

TABLE II

AMOUNT OF SAP OBTAINED, TOTAL SOLIDS, AND PER CENT. OF SUGARS IN THE TISSUE CALCULATED FROM EXPRESSED SAP ANALYSIS UNDER DIFFERENT TREATMENTS AND CONDITIONS OF OBTAINING SAP

VARIETY	TISSUE	SAP FROM 100 GRAMS OF TISSUE	TOTAL SOLIDS IN SAP	TOTAL SUGARS IN TISSUE	REDUCING SUGARS IN TISSUE	SUCROSE IN TISSUE	SIZE OF SAMPLE	PRESSURE PER SQUARE INCH	TREATMENT OF TISSUE
		gm.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*gm.*	*lb.*	
Burr-Leaming	Leaf	62.0	9.7	2.98	.....	.....	25	10000	Ground
"	"	59.8	9.6	3.00	.....	.....	50	10000	"
"	"	57.3	9.4	2.98	.....	.....	100	10000	"
"	"	55.5	9.3	2.94	.....	.....	150	10000	"
"	"	54.7	9.4	2.90	.....	.....	200	10000	"
Burr-Leaming	Leaf	55.7	10.3	2.57	.....	.....	100	1000	Ground
"	"	59.4	10.5	2.55	.....	.....	100	5000	"
"	"	59.4	9.7	2.45	.....	.....	100	10000	"
"	"	60.6	9.9	2.57	.....	.....	100	14000	"
Burr-Leaming	Stem	79.4	12.4	9.01	.....	.....	100	1000	Ground
"	"	80.8	12.4	8.94	.....	.....	100	5000	"
"	"	82.2	12.4	8.99	.....	.....	100	10000	"
"	"	82.2	12.4	8.94	.....	.....	100	14000	"
Burr-Leaming	Leaf	57.5	9.4	2.56	1.43	1.07	100	10000	Ground
"	"	57.5	9.1	2.55	2.31	0.24	100	10000	Ground and frozen
"	"	.....	.....	2.56	1.45	1.05	100	.....	Alcoholic extraction of tissue
Burr-Leaming	Stem	80.9	10.7	9.11	2.33	6.49	100	10000	Ground
"	"	81.8	10.4	9.27	2.40	6.53	100	10000	Ground and frozen

was used as the unit on which the measurements were based. It was desirable to see what effect different treatments, size of samples, and pressure would have on the results. Data which were obtained by using different sized samples in the press cage, other conditions remaining the same, are given in table II. These data show that, although the proportion of sap expressed depends on the size of the sample, a greater percentage being removed from the smaller samples, the sugar content of the tissue calculated from the analysis of the expressed sap is practically the same for each sized sample. The data, when the pressure was varied, are also given. Pressures of over 5000 pounds per square inch did not remove proportionally larger amounts of sap, and the sugar content of the sap obtained from tissue under higher pressures is not affected. Since much more trouble is encountered in using higher pressure, 5000 pounds is recommended as a convenient standard.

In some of the experiments reported by other investigators, plant tissues were frozen before expressing the sap. In a former paper (**6**) it was noted that freezing corn tissue did not result in a very much larger yield of sap than grinding; thus the latter operation was adopted as being more convenient. The possibility that freezing might change some of the properties of the sap also was considered. Some evidence on this point has been obtained in studying the effect on sugar content of freezing ground tissue at –25° C. The results are shown in table II. The amount of sap obtained and the total solids in the sap from the frozen and unfrozen tissue are not importantly different. The total sugar contents of the tissue, as estimated from the expressed sap from the frozen and unfrozen tissue, also agree well with each other and with that determined by alcoholic extraction. The sucrose in the leaf tissue, on the other hand, was much lower after freezing, indicating that hydrolysis had occurred. Similar results were obtained a number of times. Slight hydrolysis occurred in the stem tissue also, but not as much as in the leaf tissue. Total sugars, therefore, can be obtained from tissue which has been frozen, but the true sucrose content of leaf tissue can be obtained only from tissue not frozen. These results are in accord with those of MASON and MASKELL (**4**), who found an apparent inversion of sucrose in the sap of cotton leaves after freezing at – 16° C. for 24 hours.

Discussion

On the basis of the results reported, it appears that when corn tissues are ground and pressed under the conditions described, the first portions of the expressed sap are obtained, for the most part, from the broken cells in the mass of ground tissue and, consequently, there is included a certain amount of colloidal protoplasmic material. As the pressure is increased

the mass of tissue is compressed and begins to exert a filtering action, filtering out the larger colloidal particles. As the pressure is increased further the sap, which now is expressed for the most part from unbroken cells, is filtered to a still greater extent, so that the last portion contains a noticeably smaller amount of colloidal material than the first portion.

That this is true is indicated by the fact that the determinations which included colloidal materials, as well as those in true solution, such as total solids, total nitrogen, and total phosphorus, showed a progressive decrease with each successive portion. Materials in true solution, such as sugars, nitrates, inorganic phosphates, and such measurements as conductivity, freezing point depression, and H-ion concentration, were constant in the successive portions of the sap.

Since there is no reason to believe that grinding of the tissue and subsequent pressing out of the sap has influenced the relationship between these solutes and the solvent to any extent, it may be assumed that the concentration of the sap which has been expressed is similar to that which is retained in the press cake so far as the concentration of the solutes is concerned. It follows then that from determinations of the concentration of any solute in the solvent in the expressed sap and the total quantity of the solvent in a given sample of the tissue, the total quantity of the solute in the tissue can be calculated.

Summary

1. The procedure for calculating the total sugar content of corn tissue from analysis of expressed sap is described in some detail.

2. The total sugar, reducing sugars, and sucrose content of corn tissue can be calculated from analysis of the expressed sap just as accurately as they can be determined by alcoholic extraction of the tissue.

3. The total sugar content is not affected by the size of the sample, the pressure used, nor the preliminary treatment.

4. The sucrose content of corn leaf tissue is decreased by freezing the tissue but is not affected by grinding it.

5. It is concluded that the composition of corn tissue with respect to any constituent in true solution in the sap can be determined from an analysis of the expressed sap.

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CHANGES IN THE COMPOSITION OF THE GARDEN PEA AFTER HARVEST

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Introduction

In this paper data are presented showing some of the changes that occur in the fruit of the garden pea after harvest, when held at different temperatures. The object of this study was to determine the changes in at least a few of the components that were thought to be so altered after harvest as to affect the edible quality. In this study the changes in only a few of the carbohydrates were determined, but no doubt a large number of other chemical as well as structural changes occur that are to some extent responsible for the rapid loss of edible quality when held under certain conditions after harvest.

Experimental procedure

The variety of pea used was the Dwarf Telephone. This is a large-podded wrinkled pea, and is the one used in the Imperial Valley for shipping in the early spring. The work was started in 1925, and the material studied that year was harvested from the same field and from the same vines as used for the study of pea development previously reported (**1**). Open flowers were labeled on April 9, 1925, and the fruit of these tagged flowers was in the best stage for harvest on May 9. The fruit was harvested between 7:30 and 8:00 A. M. and placed in storage at once at 0, 5, 14, 25, and 35° C. In 1925 small chambers were used for storage at 25 and 35° C. In these the rapid loss of water from the fruit caused a high relative humidity and the surface of the pods remained moist. The combination of high humidity and high temperature made a favorable environment for bacterial activity and the pods started to decay after two days at 35° C. and after three days at 25° C. For this reason the data for these two high temperatures were discarded. During the entire storage period at both 0 and 5° C. the fruit remained green and turgid.

The work was repeated again in 1927 at the three higher temperatures. Much larger samples were used than in 1925, and these were stored in large constant temperature rooms, in thin layers in shallow trays with slatted bottoms, to facilitate aeration and prevent heating. Samples were removed every 24 hours, shelled, and the pods and peas preserved separately. The methods of preserving and analyzing samples were the same as those previously used (**1**) in the developmental studies of the fruit.

Experimental results

Determinations were made for dry matter content, reducing sugars, sucrose, starch, acid-hydrolyzable polysaccharides, and crude fiber for both the 1925 and 1927 series.

Composition of pods and peas

The results of the analyses of fruit stored at different temperatures are presented in tables I to VI. The initial composition of the pods or peas for the same component differed somewhat for the two years. This may have been due partly to environmental conditions, but was probably due mainly to the fact that there was a difference in the stage of maturity of the fruit at time of harvest. The higher percentage of dry matter and starch and the lower percentage of sucrose of the peas seem to show that the average fruit was slightly more mature in 1925 than in 1927.

Dry matter

As shown in table I, the *pods* of the fruit stored at 0–14° C. remained

TABLE I

Percentage of dry matter in the pods and peas

Hours in storage	Storage temperatures				
	1925 Series		1927 Series		
	0° C.	5° C.	14° C.	25° C.	35° C.
	Pods				
0	14.34	14.34	14.09	14.09	14.09
24	14.87	14.86	12.83	13.22	13.16
48	14.81	14.74	12.32	13.26	13.03
72	14.80	13.17	12.73	13.13	13.22
96	14.98	15.13	15.56	13.14	15.59
120	14.78	14.53	13.15	15.45	20.67
144	13.28	13.12	.....	.....	.....
168	.....	13.57	.....	.....	.....
	Peas				
0	23.01	23.01	21.69	21.69	21.69
24	23.02	23.37	22.02	22.62	23.25
48	23.67	24.66	22.39	24.48	23.55
72	23.44	24.34	23.29	24.54	24.02
96	23.42	25.58	23.49	25.07	25.26
120	23.40	25.96	24.19	26.62	27.14
144	23.18	25.96	.....	.....	.....
168	23.63	25.67	.....	.....	.....

rather constant in percentage of dry matter throughout the storage period. There was an increase at 25° C. and a somewhat greater increase at 35° C. This increase in the percentage of dry matter is probably due almost entirely to loss of water from the pods by transpiration. In the *peas* stored at 0° C. the percentage of dry matter remained almost constant, while at the higher temperatures it increased. For a time after harvest the water lost from the pods by transpiration is probably replaced by a withdrawal of water from the peas, causing the percentage of dry matter of the peas to increase before there is any noticeable change in the percentage of dry matter in the pods.

Sugars

As shown in table II, the percentage of reducing sugars in the *pods*

TABLE II

Reducing sugar in pods; expressed as percentage of dry weight

Hours in storage	Storage temperatures				
	1925 Series		1927 Series		
	0° C.	5° C.	14° C.	25° C.	35° C.
0	13.69	13.69	12.15	12.15	12.15
24	.....	.....	12.25	11.38	9.80
48	12.31	13.11	12.06	11.85	9.60
72	12.72	13.99	12.42	9.32	8.45
96	13.64	.....	10.60	8.31	4.30
120	.....	12.71	10.01	5.53	1.66
144	14.18	12.68	.....	.....	.....
168	.....	13.48	.....	.....	.....

remained rather constant during the entire storage period at both 0 and 5° C., but at 14° C. there was a somewhat rapid decrease after 72 hours. At both 25 and 35° C. there was considerable loss in the percentage of reducing sugars, but the rate of loss was much more rapid at 35° C. The *peas* of the fruit showed a trace of reducing sugars after being stored 120 hours at 0 and 5° C., but there was no trace of them at the higher temperatures.

Table III shows that there was a rather regular loss in the percentage of sucrose in the *pods* at all temperatures, the rate of loss increasing with the temperature. In the *peas* stored at 0° C. the percentage of sucrose remained almost constant for the entire storage period. At 5, 14, and 25° C. the rate of loss increased as the temperature was raised. At 25° C. about 34 per cent. of the sucrose was lost during the first 24 hours, and

TABLE III

SUCROSE IN PODS AND PEAS; EXPRESSED AS PERCENTAGE OF DRY WEIGHT

HOURS IN STORAGE	STORAGE TEMPERATURES				
	1925 SERIES		1927 SERIES		
	0° C.	5° C.	14° C.	25° C.	35° C.
	Pods				
0	13.89	13.89	14.35	14.35	14.35
24	.....	.....	11.02	9.79	8.67
48	13.79	.....	9.15	8.04	5.68
72	12.27	11.05	7.14	5.79	3.90
96	10.71	.....	6.70	4.01	1.90
120	.....	9.63	5.62	2.30	1.24
144	9.51	7.61	.....	.....	.....
168	.....	7.88	.....	.....	.....
	Peas				
0	22.67	22.67	28.55	28.55	28.53
24	24.44	22.58	24.14	17.85	18.22
48	22.04	20.88	19.37	10.91	12.12
72	.....	.....	14.54	7.16	8.81
96	22.29	19.53	11.66	5.19	5.54
120	21.83	17.49	9.89	4.08	4.35
144	.....	.....	.....	.....	.....
168	23.36	16.72	.....	.....	.....

after 5 days only about 14 per cent. of the original amount of sucrose remained. At 35° C. the rate of loss was slightly lower than at 25° C.

POLYSACCHARIDES

In the *pods* there was a reduction in the percentage of starch at all temperatures, the rate of loss increasing as the temperature was raised up to 25° C. (table IV). At 35° C. the rate of loss was about the same as at 25° C. In the peas stored at 0° C. there was very little change in the percentage of starch throughout the entire storage period. There seemed to be a slight increase up to 72 hours, then a slight decrease. In the peas stored at temperatures above 0° C., however, there was a definite increase in the percentage of starch. During the first 24 hours the rate of increase in the percentage of starch was most rapid at 35° C., but after the first 24 hours the rate of increase was most rapid at 25° C. The 35° C. temperature seemed to inhibit, to a certain extent, the formation of starch.

In the *pods* of the fruit stored at 0° C. the percentage of acid-hydrolyzable polysaccharides (starch deducted) did not seem to show any definite

TABLE IV

STARCH IN PODS AND PEAS; EXPRESSED AS PERCENTAGE OF DRY WEIGHT

Hours in storage	Storage temperatures				
	1925 Series		1927 Series		
	0° C.	5° C.	14° C.	25° C.	35° C.
	Pods				
0	6.63	6.63	7.98	7.98	7.98
24	7.11	6.57	5.59	4.37	5.25
72	5.94	6.13	4.30	2.74	2.52
120	5.51	5.47	2.85	1.59	1.83
144	5.10	.....	.....	.....	.....
168	.....	3.93	.....	.....	.....
	Peas				
0	16.06	16.06	12.36	12.36	12.36
24	16.27	17.23	14.15	17.56	18.93
48	.....	.....	.....	22.00	21.22
72	17.51	18.22	18.73	24.21	22.66
120	16.97	18.22	20.58	23.40	21.85
144	15.38	.....	.....	.....	.....
168	15.68	20.06	.....	.....	.....

TABLE V

ACID-HYDROLYZABLE POLYSACCHARIDES (STARCH DEDUCTED) IN PODS AND PEAS; EXPRESSED AS PERCENTAGE OF DRY WEIGHT

Hours in storage	Storage temperatures				
	1925 Series		1927 Series		
	0° C.	5° C.	14° C.	25° C.	35° C.
	Pods				
0	13.26	13.26	13.89	13.89	13.89
24	12.81	14.42	14.66	15.24	13.26
72	14.01	14.04	15.25	18.03	17.35
120	15.02	14.71	17.00	21.88	20.66
144	13.22	.....	.....	.....	.....
168	.....	16.86	.....	.....	.....
	Peas				
0	10.87	10.87	10.53	10.53	10.53
24	10.17	10.03	12.13	12.72	10.08
48	.....	.....	.....	12.62	12.21
72	11.34	13.08	11.96	13.23	14.21
120	11.32	13.02	14.85	14.18	14.14
168	10.41	12.85	.....	.....	.....

trend; at 5° C., however, there was a rather irregular but definite increase (table V). The most rapid increase in the percentage of acid-hydrolyzable polysaccharides in the pods occurred at 25° C. At 35° C. the rate of increase was less than 25° C. In the *peas* the percentage of acid-hydrolyzable polysaccharides remained rather constant at 0° C. At 5° C. there was a considerable increase during the first 72 hours of storage, but there was very little change thereafter. At 14, 25, and 35° C. there was a rather irregular increase in acid-hydrolyzable polysaccharides, and after 120 hours of storage there was very little difference in the amount that accumulated at these three temperatures.

TABLE VI

CRUDE FIBER IN PODS AND PEAS; EXPRESSED AS PERCENTAGE OF DRY WEIGHT

HOURS IN STORAGE	STORAGE TEMPERATURES				
	1925 SERIES		1927 SERIES		
	0° C.	5° C.	14° C.	25° C.	35° C.
	Pods				
0	14.96	14.96	14.30	14.30	14.30
24	14.77	15.29	15.75	16.52	16.86
72	15.68	15.94	11.76	19.24	20.79
120	16.34	16.37	18.62	23.87	25.97
144	15.82	.....	.....	.....	.....
168	.....	18.16	.....	.....	.....
	Peas				
0	8.37	8.37	8.41	8.41	8.41
24	8.29	8.41	10.07	10.85	10.19
48	.....	.....	.....	11.32	10.61
72	8.38	8.55	10.86	11.28	11.35
120	8.41	8.62	11.17	10.93	11.05
168	8.46	8.55	.....	.....	.....

As shown in table VI, there was an increase in the percentage of crude fiber of the *pods* at all temperatures. The smallest increase occurred at 0° C. and the greatest at 35° C. In the *peas* held at 0 and 5° C. the percentage of crude fiber remained almost constant throughout the entire storage period. At 14, 25, and 35° C. there was a small initial increase, after which the percentage of crude fiber remained somewhat constant. There was very little difference in the percentage of crude fiber of the peas after 120 hours of storage at the three high temperatures.

TABLE VII

COMPOSITION OF PODS AND PEAS STORED SHELLED AND UNSHELLED AT 25° C., 1925 SERIES; COMPONENTS EXPRESSED AS PERCENTAGE OF DRY WEIGHT

HOURS IN STORAGE	DRY MATTER IN PRESERVED MATERIAL		REDUCING SUGARS		SUCROSE		STARCH		ACID-HYDROLYZABLE POLYSACCHARIDES (STARCH DEDUCTED)		CRUDE FIBER	
	UNSHELLED	SHELLED	UNSHELLED	SHELLED	UNSHELLED	SHELLED	UNSHELLED	SHELLED	UNSHELLED	SHELLED	UNSHELLED	SHELLED
	Pods											
0	14.09	14.09	13.69	13.69	13.88	13.88	6.63	6.63	13.26	13.26	14.96	14.96
6	.....	15.75	.....	12.00	.....	14.04	.....	.....	.....	.....	.....	.....
12	13.66	15.01	12.55	12.41	12.27	14.20	.....	6.28	.....	13.50	.....	15.14
24	13.74	17.49	12.42	12.01	11.07	11.53	6.69	4.28	13.36	13.01	16.39	15.81
	Peas											
0	23.50	23.50	.....	.....	22.67	22.67	16.06	16.06	10.87	10.87	8.36	8.36
6	.....	23.41	.....	.....	.....	19.89	.....	.....	.....	.....	.....	.....
12	22.23	22.78	.....	.....	20.82	18.62	.....	19.78	.....	9.93	.....	8.77
24	23.24	22.92	.....	.....	16.18	10.73	19.67	22.60	11.14	11.06	8.91	9.19

COMPARISON OF PODS AND PEAS STORED SHELLED AND UNSHELLED

In 1925 tests were made to compare the rate of change of some of the carbohydrate components in pods and peas when these were stored shelled and unshelled. The shelling was done immediately after harvest and the pods and peas were held at 25° C. Unshelled fruit was stored in the same chamber with the shelled. Samples were removed for analyses after 6, 12, and 24 hours.

As shown in table VII, the percentage of dry matter increased much more rapidly in the *pods* when they were stored shelled, probably because when the pods were broken the water had an opportunity to evaporate from the inner as well as the outer surface. The percentage of dry matter in the *peas* remained rather uniform throughout the 24 hours of storage. It is entirely likely that the high respiratory activity during this early period produced sufficient water to compensate for that lost by evaporation.

There was some loss in the percentage of reducing sugars and sucrose of the *pods* in both the unshelled and shelled series. In the *peas* the loss in percentage of sucrose was very rapid, a considerable drop having taken place even after six hours of storage. There was a marked difference in the rate of loss of sucrose between the two series, the percentage being reduced at a much more rapid rate in the shelled peas than in the unshelled. At the end of 24 hours about 53 per cent. of the sucrose had been lost in the shelled peas, while only about 29 per cent. had been lost in the unshelled. In the 1927 series (table III) the peas stored unshelled lost 37.5 per cent. of their sucrose during the first 24 hours of storage at 25° C. The initial percentage of sucrose, however, was considerably higher in the 1927 series, which may account for the more rapid loss. From this it would appear that the peas should not be shelled until ready for use, especially if they are to be held at ordinary room temperature. It would indicate also that the practice of shelling peas for market, as is sometimes done, is not a good one.

The data in table VII also indicate that a rapid decrease in the percentage of starch occurred during the 24 hours in the *pods* that were stored shelled. The percentage of starch in the pods stored unshelled, however, remained rather constant; but this does not agree with the data obtained in 1927, which show that the percentage of starch in the pods decreased from 7.98 to 4.37 during the first 24 hours (table IV). In the *peas* there was an increase in the percentage of starch in both the shelled and unshelled series, but the increase was much greater in those that were stored shelled.

There was very little change in the percentage of acid-hydrolyzable polysaccharides of the *pods* in either the shelled or unshelled series. In the 1927 series, however (table V), there was a definite increase in the per-

centage of these components during the first 24 hours. There was very little change in the acid-hydrolyzable polysaccharides of the *peas* during the first 24 hours. The small change that occurred seemed to indicate an accumulation, and is approximately the same for both the shelled and the unshelled series.

There was a slight increase in the percentage of crude fiber in the pods and peas of both the shelled and unshelled series.

Discussion

One of the most important aims of the grower and shipper of peas is to have the product reach the consumer in a condition of high edible quality. Providing harvesting is done at the proper time, probably the most important factor influencing quality is the temperature at which the product is held after picking. The preceding data show that in the carbohydrates studied there are no important changes taking place when the peas are stored at 0° C. In other words, at 0° C. these components remain in about the same proportion as they occurred at time of harvest. Cooking tests after a week at this temperature showed that the peas still retained their original fine flavor. BROWN (**2**), working with the variety Telephone, states that peas held for 40 days at 0° C. still had an excellent quality when cooked.

In the peas held at temperatures above 0° C. there is a loss in the percentage of the more mobile carbohydrates and an increase in the percentage of the more stable carbohydrates. These changes greatly affect edible quality, and appear to occur most rapidly at about 25° C. BROWN (**2**) states that when peas were stored at 10° C. they still had a good flavor on the fifth day, but declined to fair and poor on the seventh and ninth days; and when stored at 27° C. they were worthless after three days.

In most long-distance shipments of peas, ice is placed directly in the container. This keeps the material at approximately 0° C., and according to the preceding data, practically all of the important carbohydrate changes in the peas are held in abeyance while this temperature is maintained. In peas shipped long distances, therefore, the changes that affect quality unfavorably must occur before the peas are packed or after they are removed from the container. In order to place a product of high edible quality upon the table of the consumer, it is therefore necessary that the peas be put into the iced container as quickly as possible after harvest, and that they be held at a low temperature after they reach their destination at the terminal markets and in the homes of the consumer.

It is interesting to note that in the *pods* there is a decrease in starch while in the *peas* there is an increase in starch at the higher temperatures. The decrease in the percentage of starch in the pods can probably be ac-

counted for to some extent by the increase in acid-hydrolyzable polysaccharides other than starch and by the increase in crude fiber. In the peas the increase in starch is probably due chiefly to the condensation of sugars. From the data at hand it is impossible to tell whether there is a movement of carbohydrates or other components from the pods to the peas after harvest.

Summary

PODS.—The percentage of *reducing sugars* in the pods remained somewhat constant at 0 and 5° C., but at the higher temperatures there was a gradual loss; the rate of loss was most rapid at 35° C. The percentage of *sucrose* decreased at all temperatures from 0 to 35° C.; the rate of loss was most rapid at 35° C. The percentage of *starch* decreased at all temperatures; the smallest loss occurred at 0° C. The losses at 25 and 35° C. paralleled each other rather closely. The percentage of *acid-hydrolyzable polysaccharides* changed but little at 0° C. The rate of increase was somewhat slower at 35 than at 25° C. The percentage of *crude fiber* increased at all temperatures. The rate of increase was least at 0 and highest at 35° C.

PEAS.—The percentage of *sucrose* in the peas remained somewhat constant at 0° C. during the entire storage period. The most rapid loss occurred at 25° C. The percentage of *starch* changed but little at 0° C., but there was a gradual increase at the higher temperatures. The decrease was most rapid at 25° C. The percentage of *acid-hydrolyzable polysaccharides* remained rather constant at 0° C. and increased but slightly at 5° C. At 14, 25, and 35° C. there was a rather irregular increase, but after 120 hours the percentage present was about the same in the three high temperature lots. The percentage of *crude fiber* remained about constant at 0 and 5° C. It also remained rather constant at the higher temperatures after a small initial increase.

FRUIT STORED SHELLED AND UNSHELLED.—In peas stored shelled and unshelled at 25° C., the most significant changes were the decrease in the percentage of sucrose and the increase in the percentage of starch. The sucrose decreased and the starch increased at a much more rapid rate in the shelled than in the unshelled peas.

The writers wish to express their appreciation to H. W. ALLINGER, of the Division of Chemistry, who made most of the analyses; also to J. G. SEWELL, of the Division of Chemistry, W. H. SPRAGUE, and MISS E. HARROLD, who helped prepare the material for analysis and did a portion of the analytical work.

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SEASONAL SHRINKAGE OF MONTEREY PINE AND REDWOOD TREES

FERDINAND W. HAASIS

(WITH FOUR FIGURES)

Introduction

It has for some time been a matter of observation that Monterey pine trees growing at Carmel, California, show a notable increase in diameter in the late fall or early winter months, apparently preceding the beginning of new spring growth. MACDOUGAL writes of one pine tree, for example, in which growth had ceased in August, as showing in late November and again in December "slight impulses of enlargement which are characteristic of the species in this region" (**10**, p. 10). MACDOUGAL (**7**, pp. 27–37; **8**, pp. 342–343) and LODEWICK (**5**), moreover, have reported slight shrinkages in the diameter of trees of several species at various seasons, but without attempting to correlate their observations very closely with external conditions.

Records of such phenomena have been obtained by means of the dendrograph, an instrument devised by MACDOUGAL in 1918 (**7**, pp. 10–15). This instrument may be described briefly as consisting of a rigid frame constructed of material with a low coefficient of expansion, suitably supported about a tree, and carrying two contact rods, one fixed and the other

FIG. 1. Dendrograph in position on a pine tree, showing floating frame, contact rods, lever assembly, recorder, and supports.

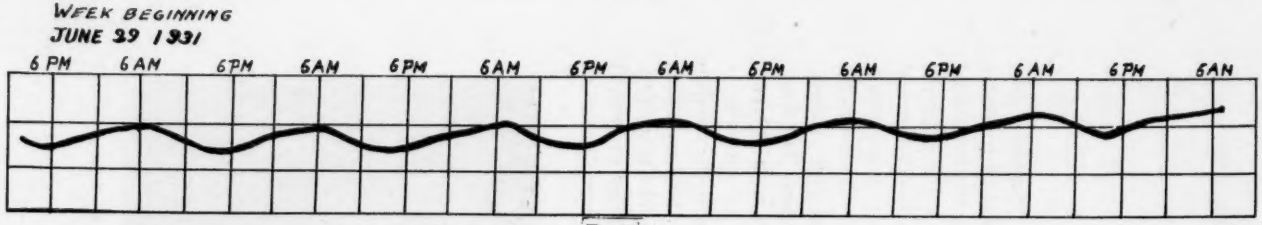

FIG. 2

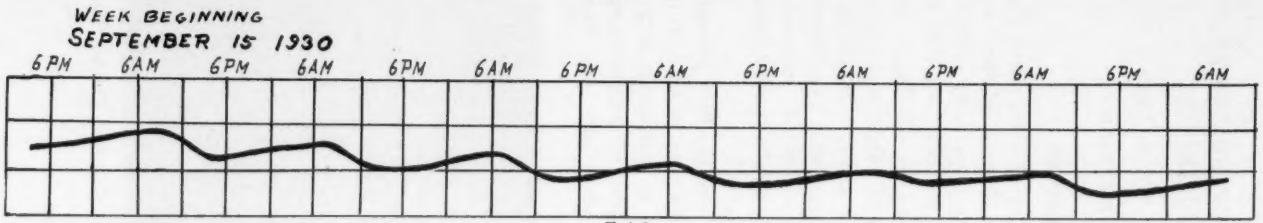

FIG. 3

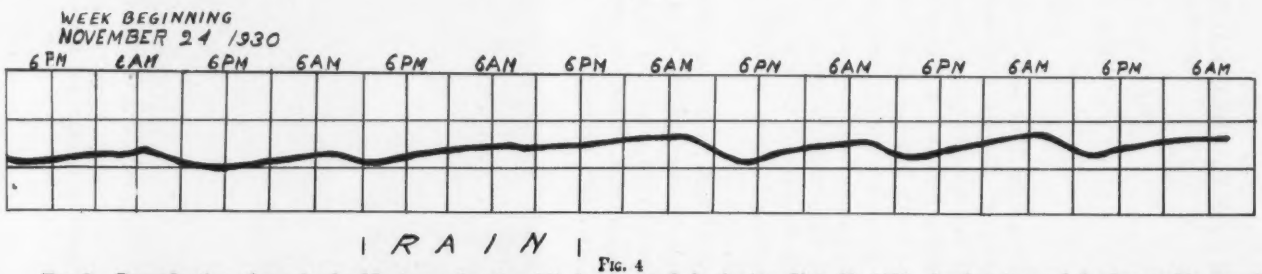

FIG. 4

FIG. 2. Reproduction of graph of a Monterey pine (no. 33) for the week beginning June 29, 1931, showing type of dendrographic record made by many kinds of trees in clear weather during growing season. Spaces between vertical lines represent 6-hour periods, between horizontal lines 10-mm. intervals on the sheet; ×11.

FIG. 3. Dendrographic record of a Monterey pine (no. 1–A) during period of progressive shrinkage in the fall of 1930. Daily reversible variations in diameter are shown, like those in figure 2, but the net change in diameter for the week is a decrease rather than an increase. Spaces between vertical lines represent 6-hour intervals, between horizontal lines 10-mm. intervals on the sheet; ×11.

FIG. 4. Dendrographic record of a Monterey pine (no. 1–A) showing reversal of diametral change in a week (November 24–December 1, 1930) when rain interrupted a period of dry weather. Rain began about noon of November 26 and continued through most of the following day, a total of 0.90 in. (23 mm.) falling; ×11.

movable. The movable rod actuates a pen lever, by means of which a continuous record of changes in diameter is made on a ruled sheet borne on a revolving cylinder. The general appearance of such an instrument is illustrated in figure 1. The direction of movement on the record sheet is at right angles to that of the movement between contact points.

The dendrographic records show that ordinarily, in clear weather, trees shrink during the day and swell at night. A week's graph, therefore, appears as a sinuous line. When growth is taking place, each successive nocturnal maximum is higher on the record sheet than the preceding one. A record of this type can be regarded as a normal graph for a growing tree. An example of this kind of graph is reproduced in figure 2.

Observations and discussion

Seasonal variations

The dendrographic records upon which this paper is based were made at Carmel, a short distance from Monterey, in Monterey County, California, and in the canyon of Rocky Creek, about 17 miles (27 km.) south of Carmel. Carmel is situated on a slight bay open to the Pacific Ocean. The trees under observation are about half a mile (1 km.) from the beach, located partly on top of an old fixed dune, partly on the northeast slope of this dune, and partly at its foot. The soil is markedly sandy. The trees at Rocky Creek are for the most part about a mile (1.5 km.) from the ocean, in a relatively narrow canyon. Here the soil is mainly residual, from granitic rock.[1]

In general, the climate of this region (which lies at about 36° N) is marked by a winter rainy season and a summer dry season. The latter can be expected to begin in May or June. It commonly lasts through August or September and may extend later, although not always absolutely without measurable precipitation for the entire period. The annual precipitation, on the basis of 21 years' records, varies from 9 to 27 inches (24–69 cm.), averaging 17 inches (43 cm.).

In the season of least precipitation, ocean fogs frequently cover the region, and as a result temperatures tend to be lower than in places farther inland. The combination of high atmospheric humidity and low temperatures greatly reduces the evaporating power of the air for much of the summer period. Frosts occur occasionally, both at Carmel and in Rocky Creek canyon, but for the most part the winters are relatively mild. On the whole the climate is comparatively equable, the extreme temperatures recorded in an instrument shelter on the fixed dune at Carmel ranging

[1] For detailed descriptions of the ecological features of this region see Shreve (13, 14).

1930) when rain interrupted a period of dry weather. Rain began about noon of November 26 and continued through most of the following day, a total of 0.90 in. (23 mm.) falling; ×11.

from 18° F. (−8° C.) in January, 1922, to 101° F. (38° C.) in October, 1917.

A detailed examination of the dendrographic records shows that an appreciable amount of shrinkage regularly occurs during the autumn prior to the fall or winter swelling which has been noted. This of course is especially marked in years when unusually long dry periods occur. Examples of such notably long dry periods in summer and autumn are afforded by the years 1926, 1929, and 1930. The records of several trees on the dune at Carmel show more or less continuous shrinkages during these essentially rainless periods. Typically these decreases in diameter begin in the latter part of the dry period; and with the advent of rain, diametral increases commonly occur.

These conditions are illustrated in figures 3 and 4. Figure 3 shows the kind of graph produced in September when a pine tree was shrinking. In figure 4 the effects are shown of a 0.9-inch rainfall occurring on the 26th and 27th of the month. It will be observed that the tree increased appreciably and rather steadily in diameter during this 2-day period, but again showed pronounced diurnal fluctuations on succeeding days.

Exceptions to this general program occur, which are apparently to be correlated with differences in soil moisture supply. For instance, one vigorous tree, situated in a swampy location at the base of the hill, did not begin to contract in 1929 until mid-November; whereas contraction in those on the dune in general had commenced at least a month earlier.

There also occur individual differences among the trees on the dune, one tree continuing to grow, for example, after others have begun to shrink. These divergencies can be ascribed partly to differences in root development of the several trees. In addition there appear to be pockets of moister soil here and there on the dune, where conditions for tree growth are more favorable than elsewhere. An irregular layer of hardpan formed of cemented sand is found at a depth of about 1 meter below the top of the soil. The upper surface of this hardpan is very irregular, and it seems likely that over a moderately extensive area there may be developed basins in which the moisture supply is considerably more abundant than at other, near-by, places. Trees which happen to start their growth over such pockets will be better able to continue growth during dry weather than those not so situated.

The records for the redwoods are less complete than are those for the pines, generally having been discontinued in the fall and resumed the following spring. Nevertheless the same general sort of seasonal shrinkage is shown. For example, in 1926 one tree, situated on a slope with southerly exposure, shrank appreciably in September and October. Another, on a north slope where moisture conditions were presumably more

favorable, did not exhibit marked shrinkage until the latter part of October.

During the winter of 1930–1931, most of the dendrographs on redwoods were kept in operation throughout the season, just as were those on the pines. In this season, trees on both north and south slopes showed definite shrinkages in October, November, and December, followed by swelling early in January.

Evidently we are dealing with a drying out and a subsequent rehydration of the tree tissues. Directly after the beginning of a dry season, there will still be a certain amount of soil water available to the trees. Gradually, however, this supply becomes exhausted. In the summer or early autumn, the soil moisture content at a depth of 15–30 cm. may fall as low as 3 or 4 per cent. (on the dry weight basis) on the dune at Carmel. With the advent of rain, soil moisture again becomes available and the trees are enabled to make up the losses suffered during the dry period. It seems likely that the first diametral increases occurring after a dry period are not to be ascribed to the consequences of actual cell division, but simply to swelling of the partially dried tissues.

It should be pointed out that this seasonal shrinkage is not the same as absence of growth, but a decrease in diameter just as definite as the increases occurring during periods of cell division.

The essential identity of seasonal and diurnal variations in diameter will be shown in the succeeding section.

Conditions affecting shrinkage and rehydration

Speaking of daily reversible variations, MacDougal has mentioned the fact that "contraction or shrinkage is an invariable attendant of lessened water-balance due to an excess of transpiration over water supplied" (**9**, p. 3). It seems obvious that seasonal shrinkage is to be explained in a similar way. Progressive shrinkage occurs when the soil is so dry that it cannot supply enough moisture to the tree to make up for the transpirational losses.

Evaporating power of the air.—It is to be supposed that when the evaporating power of the air is reduced, water is absorbed and a certain amount of swelling ensues. The assumption is justified by the fact that nightly diametral increases occur during a period of progressive shrinkage, the tree, however, suffering a net loss in diameter from night to night. If, therefore, the period during which the evaporating power of the air were low could be extended, it would seem that the progressive shrinkage could be checked or reversed. While it is not practicable to lengthen the period of darkness experimentally for a large tree, yet cloudy or foggy days do

afford conditions under which the transpirational losses are somewhat reduced.

On one prevailingly cloudy day in May, several of the trees under observation shrank to a markedly less extent than they had been doing for some time past. Moreover, although shrinkage (or a static condition) had been evident for the past few nights, on this night definite increases occurred.

The question might be raised, whether the shrinkage and swelling which have been observed are due simply to drying of the thin layers of bark under the contact points of the dendrograph, and reabsorption of water from the moist air or from free water upon the bark itself. A number of observations and experiments have served to throw light on this question.

During a notably foggy night in April, several of the pines showed pronounced diametral increases as compared with the preceding night, although there had been progressive shrinkage for some time preceding. A conspicuous exception to this general performance was afforded by a small pine tree 1 cm. in diameter, in which shrinkage very definitely continued during this night. Presumably this is to be explained by considering that the roots of this small tree are not well enough developed to enable it to take in sufficient water for diametral increases, even when the evaporating power of the air is somewhat reduced. The contacts of the instrument on this tree are outside of the bark. The redwoods and the pines at the base of the dune, with ample water supplies, had been showing diametral increases prior to this foggy night, and the increased atmospheric humidity had no apparent effect upon their daily program.

It has been customary, in setting up the dendrographs on pines and redwoods, to seat the contact points on an area in which the bark has been thinned down to a thickness of about 2 mm. outside the cambium layer (MacDougal, **7**, pp. 20–21). If this layer of bark were to swell appreciably when it became moist, the dendrographic records would obviously show an increase due to this cause; and duplicate instruments with contacts on different thicknesses of bark ought to show differences in amount of such increase. There was accordingly attached to a Monterey pine tree, carrying a dendrograph with the contacts on thin layers of bark, a second instrument with the contacts on the outside of the 1.5-cm. bark, which was operated for seven weeks during January and February. While the graphs from the two instruments are not absolutely identical, week for week, yet their agreement is remarkably close. If water is absorbed to an appreciable extent from the outside, then the instrument with contacts seated on the thicker bark would be expected to record greater fluctuations with alternating periods of moist and dry air than that with contacts on the thinner bark. On the other hand, if the intact bark were resistant to

wetting while the exposed bark were not, differences in amount of fluctuation would still be expected. The conclusion accordingly emerges that swelling of the bark due to absorption of external moisture accounts for not more than a very small part of the diametral increase which is associated with rainy or foggy weather.

Further evidence along these lines is afforded by an experiment in which the bark in the vicinity of the contact points of a Monterey pine was wetted with tap water, no unusual increase in diameter resulting from this treatment. On the other hand, MACDOUGAL reports experiments in which bast and cork tissue from Monterey pines was found to swell somewhat when placed in water (**7**, pp. 37–38).

Reduction of the evaporating power of the air can also be effected by lowering the temperature. This occurs at night and during stormy weather, in the latter case being especially associated with increased atmospheric humidity. Another way in which solar radiation can be cut off, with resulting temperature reduction, is exemplified by a partial solar eclipse around mid-day, which occurred in April, 1930. This was reflected in a reversal of the diurnal shrinkage among the pine trees. In the redwoods this swelling was less marked.

The intimate relation between shrinkage and evaporating power of the air is further brought out by the performance of the trees during extremely windy weather. Following a 10-day period with considerable rain and cloudy weather in February, 1931, there was a very clear day with a northerly wind at times reaching a velocity estimated at about 35 miles an hour. A general marked increase of diurnal shrinkage, both at Carmel and at Rocky Creek, was recorded on that day. On other windy days during the spring there has been a similar tendency toward exaggerated diurnal shrinkage. In the middle of April, 1931, the graphs of several trees at Carmel showed a maximum diameter about 8 A. M. for one prevailingly foggy day, while on the following day the greatest diameter was around 6 A. M. On this second day a pronounced southwest wind came up about 6 A. M.

On the other hand, when an easterly wind died down in the middle of the morning at Carmel, this fact was reflected by a decreased rate of diurnal contraction, or in some cases by an actual increase in diameter. PHILLIPS reproduces two annotated dendrograms showing marked contraction, similar to that just noted, in hardwood trees in South Africa during periods when warm dry winds were blowing (**12**, pp. 46, 52, Pls. V, XI).

SOIL MOISTURE SUPPLY.—From what has already been said, it is obvious that the autumnal shrinkage is concurrent with a very dry condition of the soil, at least on the dune at Carmel. Judging by the low stage of Rocky Creek during the fall of 1930, it seems likely that in the case of the

redwoods as well, an inadequate water supply is responsible for the observed shrinkage. Additional evidence of the correlation between soil moisture content and shrinkage is afforded by the performance of the trees in the spring of 1931. During a period of scanty rainfall at this time, shrinkage of the trees upon the dune was observed, while those at the foot of the dune were increasing in diameter. At the end of March the moisture contents of the soil on the dune and at the foot were, respectively, 8 per cent. and 27 per cent., at a depth of 30 cm. By the first of May the moisture in the former had dropped to about 5 per cent. The trees at Rocky Creek, also, exhibited growth during this period. While no soil sampling was done there, it seems reasonable, in view of the physical conditions in this canyon, to assume that these redwood trees were receiving a somewhat plentiful supply of moisture slowly seeping down from the higher elevations of the mountains.

In July, 1920, MacDougal performed an experiment in which water was supplied to a pine tree (**7**, p. 25). At the beginning of this month it was noted that the soil was very dry and that growth in this tree had essentially ceased. During a period of four days early in the month, 15,000 liters of water were applied to the soil near the base of the tree. Diametral increase began at once and continued steadily until August 16, the period being without rain. In this case it is obvious that increase in diameter is not attributable in the smallest degree to absorption of water by the bark, from the outside.

The facts just presented lead to the conclusion that there is a very close correlation between the moisture content of the soil and the seasonal shrinkage observed in Monterey pines and redwoods. The effects, of course, are modified by such atmospheric conditions as air moisture content, air movement, and air temperature.

When desiccation has proceeded so far that there is no longer soil moisture available for making up the transpirational losses, water is apparently drawn from the plant tissues themselves, and the result is the progressive shrinkage which has been observed. Evidently these Monterey pines and redwoods are able to withstand such desiccation without injury. This ability is in rather striking contrast to that of hardwoods accustomed to summer rainfall, which in a rare summer drought were observed to suffer marked leaf loss (Hursh and Haasis, **4**).

Pearson (**11**) recorded in 1924 that western yellow pine makes its greatest growth in both height and diameter during the driest month of the year. In this case, however, soil moisture seems adequate; and temperature is, perhaps, the controlling factor.

Other factors.—Brown (**2**, p. 237) has recorded observations of an early spring swelling as follows: "Growth in white pine [*Pinus strobus*]

is divisible into (a) growth without cell division and (b) growth with cell division. The first begins as early as March [in the vicinity of Ithaca, New York] and the elements concerned (phloem) increase in radial diameter from 50 to over 100 per cent. The awakening of growth is due apparently to the rise of soil water with an accompanying increase in temperature." This writer speaks also of phloem contraction, after the end of September, which he attributes to extremely cold winter temperatures. BROWN's studies were based on small blocks of phloem and xylem, 0.5 inch long and 0.25 inch wide, cut from the tree at frequent intervals (BROWN, **1**; LODEWICK, **6**, p. 14).

In view of what has just been said, it is well to question BROWN's inclusive use of the term "growth." It seems that his "growth without cell division" could more properly be referred to as "rehydration," reserving the term "growth" for his "growth with cell division." The extremely cold temperatures which he cites are not experienced in the Carmel region; and such conditions, therefore, cannot afford the explanation for the observed shrinkage here. The question, whether soil desiccation is as important as cold in New York, also cannot be answered without further study.

HALL (**3**), working at a latitude of about 35° S, noted that eight deciduous trees of four species showed a diametral decrease in May, apparently synchronous with the autumnal leaf fall. Eight evergreens, including one species of pine, did not show this decrease. HALL presents meteorological figures which show that May did not have the lowest mean temperature for the year, and that the precipitation was slightly above the monthly average, the 49-inch (124-cm.) rainfall being distributed rather uniformly throughout the year. In this case, of course, it is possible, as HALL suggests, that there was a definite relation between leaf fall and shrinkage. It can be assumed that the evergreens did not show the shrinkage because the soil moisture content did not become critically low.

Mr. A. D. LINDSAY, of the Australian Commonwealth Bureau of Forestry, has reported, in a conversation, somewhat similar shrinkages (or "decrements") in Monterey pine. These shrinkages appeared at certain seasons of the year in plantation trees, measurements being made monthly. There is no pronounced dry season in the region where these trees are growing, and the shrinkage cannot at present be definitely correlated with environmental conditions.

LOCALIZATION OF SHRINKAGE

In the case of a tree to which two dendrographs are attached, one 6.5 meters higher than the other, it is found that, in general, swelling or shrinkage takes place at about the same time at both heights. Occasionally, however, it happens that one of these instruments will show shrinkage or swell-

ing while the other shows the reverse. The reason for these divergencies has not been ascertained.

Of perhaps even more interest is the performance of a small tree 1 meter in height. During the spring of 1931, the stem of this tree, at a height of 4 dm. above the ground, suffered a rather consistent shrinkage in diameter, a total of about 0.5 mm. during a period of nine weeks in March, April, and May. At the same time that this shrinkage was taking place, the terminal shoot continued to elongate, increasing in length a matter of 8 cm. or so.

From these observations it would seem that water may be drawn away from one part of a tree and used to keep turgid the cells in another part. The plant may be shrinking at one point and swelling at another. Apparently MacDougal's suggestion (9, p. 3) that the water deficit "would increase from the base of the trunk toward its summit" would not necessarily hold true under all conditions.

Another example of shrinkage in one part of a tree while another part is swelling is afforded by dendrographic studies of diametral fluctuations in different regions of the same individual tree. From such studies it appears that the heartwood of a Monterey pine may be shrinking while the tree as a whole is swelling.

Summary

1. Detailed analysis of dendrographic records indicates that the fall or winter swelling which has been observed by MacDougal in Monterey pine trees is preceded by a progressive contraction during the dry season of the summer and fall. It appears, furthermore, that such shrinkage may occur during an unusually dry spring. In either case, with the advent of rain—or sometimes in foggy weather—such changes in diameter may be checked or reversed. The fall or winter swelling is accordingly to be ascribed to rehydration of the desiccated stem, and probably is not connected with cell division. Redwood trees also are found to exhibit these seasonal variations, which are very closely related to MacDougal's previously described diurnal fluctuations.

2. Chief among the conditions affecting shrinkage and rehydration are the evaporating power of the air (dependent upon atmospheric humidity, temperature, and wind movement) and the amount of soil moisture available, the latter apparently being the more significant from the standpoint of seasonal fluctuations.

3. A tree may be shrinking in one part while it is swelling in another.

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CONTROL OF THE FLUX EQUILIBRIUM OF ELECTROCHEMICAL PROCESSES AND ELECTRIC POLARITY IN THE DOUGLAS FIR BY TEMPERATURE

E. J. LUND

(WITH TWO FIGURES)

Introduction

As an introduction to the following description of experiments on the effect of temperature on the electric polarity in the Douglas fir, it is necessary to distinguish between two distinct types of effects of temperature on the E.M.F. produced by an electrochemical system. These two types of effects depend upon whether or not the system is in thermodynamic equilibrium. The relatively simple relation between change in static equilibrium of an electrochemical system and temperature is defined by the GIBBS-HELMHOLTZ equation $E = T\frac{dE}{dT} - \frac{\Delta H}{nF}$, where E is the electromotive force, ΔH the change in heat content of the system, T the absolute temperature, n the number of electrochemical equivalents, and F the faraday.

The effect of temperature is in general relatively small and directly proportional to the absolute temperature. So far as is known, the same statement applies to the relation between temperature and a system in metastable equilibrium while it is in the metastable state. The essential feature of the metastable state is that a new state is assumed only after a certain amount of energy is added or withdrawn from the system. The "resting" or apparently "isoelectric" condition of any irritable system, such as a nerve or muscle fiber, resembles in this respect a non-living system in metastable equilibrium. If to this extent we view the phenomenon of excitation and response of a nerve or muscle as a change from a state of metastable equilibrium, without considering the unique feature of recovery of the living nerve or muscle, then we should expect to find that the effect of temperature on the P.D. at the surface (or injury P.D.) of a resting muscle cell would be small and proportional to the absolute temperature. This is just what was found, within limits of experimental error, by BERNSTEIN (1) in his original and important study of the injury potential in muscle. An instructive inorganic system is the passive iron wire model (LILLIE 2).

Any electrochemical system which at the moment of consideration expends electrical energy is obviously not in such a state of equilibrium. From this it follows that the continuous output of electric energy from polar cells and polar cell groups is derived from a system which never attains a condition of static, dynamic, or metastable[1] equilibrium in the sense

[1] For definitions of different types of equilibria, see TAYLOR (9).

of classical thermodynamics, but represents an output from a system in a constant state of flux of energy and material. I have designated the equilibrium state in such a system a *flux equilibrium* (LUND 6).

Since a system in a constant state of flux equilibrium depends for its existence upon the maintenance of both absolute and relative velocities of change in the linked processes of the system (for example, flame, whirlpool, or catenary series of chemical reactions), it follows that temperature may indirectly affect the instantaneous magnitude of the intensity factor of the energy change, for example, the E.M.F. of an electrochemical system in such a state of flux by affecting (1) the relative and (2) the absolute velocities of the individual links in the chain of reactions.[2]

In general we should expect that the temperature coefficient of the E.M.F. of such a system would be large (and often variable), provided that the effect of temperature alters the relative velocities of the individual members in the catenary series and therefore the E.M.F., since the flux concentrations of the electromotively active substances are changed. The variation of E.M.F. in the frog skin with temperature is apparently an illustration of these conditions (LUND and MOORMAN 3).

The following experiments on the Douglas fir furnish still more striking illustrations of the behavior of such electrochemical systems in living cells. In this connection it is interesting to recall the similarity between the phenomena of continuous and discontinuous output of electrical energy by living systems and, for example, the continuous output of light by bacteria and the discontinuous output of energy from the luminous organ of such a form as the firefly, in which the feature of apparent "metastability" has been added to the mechanism by the evolutionary process of specialization in function.

Effect of temperature on electric polarity of the apex

The simplest way to approach an analysis of the phenomena is by considering first the apexes of the main axis and lateral branches. Figure 1 shows a typical arrangement of the apparatus with the result shown in the curves below. In this experiment a lateral branch was cut from the tree and removed to the laboratory with a minimum of mechanical disturbance. The apex in its natural orientation with respect to gravity was inserted in the air jacket, which consisted of a round tin tube about 3.5 inches in diameter, soldered at each end to the edges of holes cut in the sides of the galvanized iron can. Two holes were provided with rubber stoppers (*h*) to permit access to the electrode contacts. The air chamber was lined with paraffined paper for electrical insulation. The electrode contacts were the

[2] The change with temperature in relative velocities of the members of the catenary series would of course be defined by the difference in their temperature coefficients at the particular temperature under consideration.

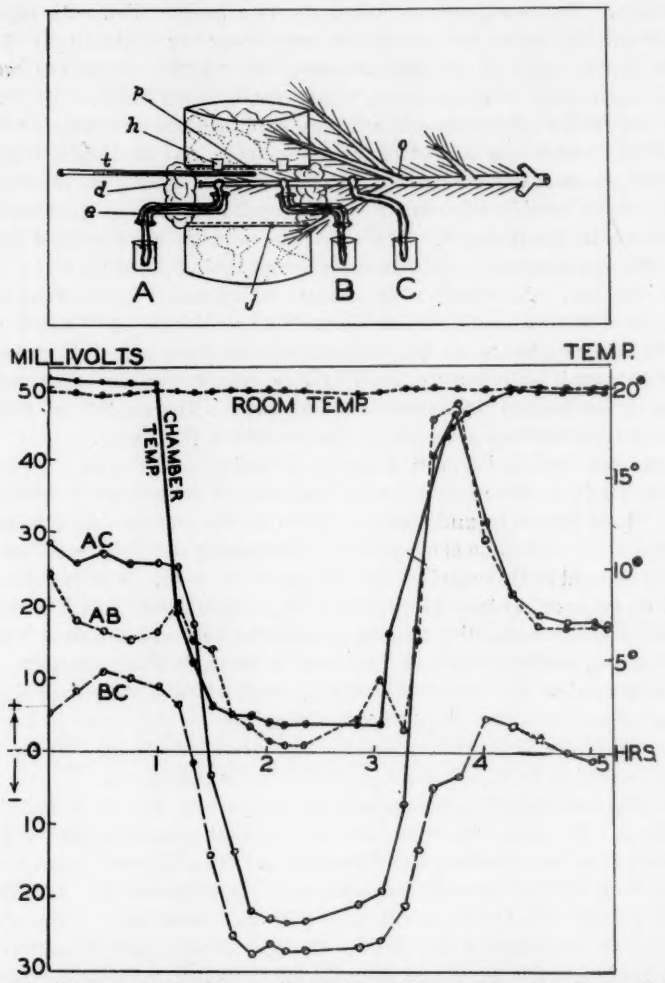

FIG. 1. Diagram at top shows sectional view of the arrangement. Open ends of the tin tube (*j*) are soldered to edges of holes cut in opposite walls of the tin vessel having a capacity of 3 gallons; heavy cotton insulation of vessel not shown. Stoppers (*h*) provide access to electrode contacts. Tin tube (*j*) is electrically insulated by paraffin paper lining (*p*); *t*, thermometer reading to 0.1° C. Dry cotton plugs (*d*) insulate the chamber. Glass tubes (*e*) contain the cotton contacts which dip into beakers with tap water, into which the Pb–$PbCl_2$ electrodes are dipped for contact. Curves *AB*, *AC*, and *BC* represent the change in magnitude and orientation of the electric polarity caused by temporarily cooling the segment *AB* from room temperature of 20 to 1.5° C.

usual cotton wicks kept saturated with tap water and inclosed in bent glass tubes (*e*). The cotton contacts dipped into a beaker filled with tap water. The electrodes, which are not shown, were hung for contact over the edge of the beakers during the measurement. Dry cotton plugs (*d*) were inserted in the ends of the chamber to prevent heat exchange. Air temperatures recorded on the graphs below were read off on a thermometer reading to 0.1° C., which was placed close to the apex. At the beginning of the experiments, tap water at room temperature was placed in the can, provided on the outside with heavy cotton insulation and an insulated cover not shown in the diagram. The electrode contacts were washed at intervals with tap water at room temperature and their P.D. checked before and after washing. As usual, insignificant variations of a millivolt or two occurred during renewal of the contacts. In most of the experiments it was found desirable to let the preparation stand at room temperature for about half an hour before readings of P.D. were begun, in order to permit effects of mechanical disturbance to disappear. The records of room and chamber temperatures are given by the curves in the figure.

Readings of P.D. between *A* and *B*, *A* and *C*, and *B* and *C* were taken systematically in the same order at intervals of from four to fifteen minutes. These values in millivolts are given in the curves. In this and following papers, the same convention of designating orientation of the polarity will be used as that employed previously (LUND **4**). For example, when the P.D. between contacts *A* and *B* is given, as in curve *AB*, it means that contact *A* is electropositive to contact *B* in the external circuit. When the curve *AB* crosses the axis, the contact *A* becomes electronegative to the contact *B*, and so on. In other words, the first letter of the pair designating the curve indicates that the electrode designated by this letter in the figure is electropositive or electronegative to the electrode designated by the second letter, according to which point on the curve is considered.

In this experiment, readings of P.D. between *A* and *B*, *B* and *C*, and *A* and *C* were taken for one hour at constant room temperature. The water at room temperature was then drained from the container, the electrodes were washed through the holes with the stoppers (*h*), and the container quickly filled with a water-ice mixture. Readings of the air temperature in the chamber and P.D.'s were continued. At the end of three hours the water-ice mixture was replaced by water at room temperature, with a continuance of the readings. The distances between the contacts *A* and *B*, and *B* and *C* were respectively 13.5 cm. and 17 cm.

The extremely interesting results are simple and clear from inspection of the curves. They may be considered as follows. The P.D. between points *A* and *B* decreases to nearly zero value at about 1.5° C., and then increases to nearly 50 millivolts, returning to the normal value of about 16

millivolts at room temperature. Since the diameter of the apex between contacts *A* and *B* is practically the same and therefore cools at the same rate, the effect cannot be due to unequal rates of cooling of the two regions, especially when we consider the long time interval involved in the experiment. The only conclusion is that the P.D. in the more apical region *A* is decreased very much more than that in the less apical region *B* by the *same* lowering of temperature. That this interpretation is correct is shown by the curve *AC*, in which only the region of contact (*A*) is cooled, resulting in a decrease in P.D. to such an extent that it becomes electronegative to the region *C*, which is maintained at constant room temperature and therefore at approximately constant electric potential. Now when we compare the simultaneous change of P.D. in the region *B* with that in *C*, as shown by the curve *BC*, it is noted that the total change of P.D. under the same conditions is less in region *B* than that in region *A*.

The second remarkable difference between the effect of temperature on the apical region *A* and that of the more basal region *B* is shown by the large "rebound" of the curves *AB* and *AC* as compared with the small "rebound" of the P.D. in region *B* in curve *BC*. It is clear that the same lowering of temperature around the apex tends to diminish the normal electric polarity; and if the lowering of temperature is sufficiently great, the electric polarity disappears and finally becomes inverted. Return to room temperature results in a return to the normal orientation of the polarity, which is followed by a great temporary increase in P.D. and then a return to normal. Experiments on the tip showed that the effects of temperature are definitely graded in their magnitude from the tip downward. This fact is further illustrated in the following experiment.

Effect of temperature on electrical polarity in a basal segment of apical internode

The diagram at the top of figure 2 shows a typical experimental arrangement when the apex of a lateral branch from near the top of a 6-year old tree was used. Contacts *D* and *C* are inside the chamber which surrounds the segment to be subjected to change in temperature. The distances between the pairs of electrode contacts *A* and *B*, *B* and *C*, *C* and *D*, *D* and *E*, and *E* and *F* are respectively 11 cm., 13 cm., 13 cm., 19 cm., and 11.5 cm. The results are shown below in the graphs, all of which are drawn to the same scale but separated into two groups for the sake of avoiding confusion in making comparisons. The curve of segment *AB*, not subjected to change in temperature, shows only a slight effect if any of temperature on its polarity by the change in the distant segment *DC*. Segment *AB* has a normal average electric polarity of about 18 millivolts. The total duration of the experiment was eight hours.

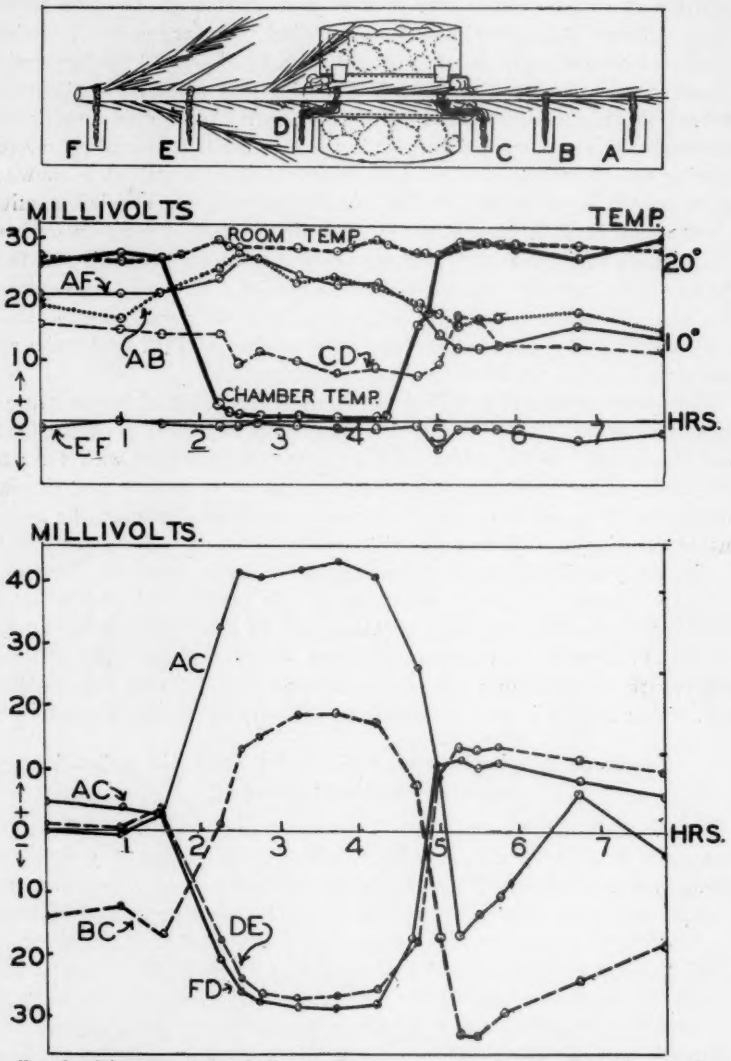

FIG. 2. Diagram at top indicates the arrangement, details of which are given in figure 1. Curves indicating the drift in E.M.F.'s of the correspondingly lettered segments are separated into two groups to facilitate comparison. The two sets of curves are drawn to same scale and may be superposed. Comparison should be made of each curve with the corresponding ones in figure 1. Lengths of the segments *AB*, *BC*, *CD*, *DE*, and *EF* are respectively 11 cm., 13 cm., 13 cm., 19 cm., and 11.5 cm.

At room temperature the polarity of the segment *BC* was *inverted.* This condition usually never occurs except after mechanical stimulation. When the temperature around *C* is lowered, its P.D. is lowered to such an extent that the electric polarity becomes oriented in the normal direction. Return to room temperature results in increase in the electropositivity of the region *C*, with the temporary "rebound" of E.M.F. This is another illustration of the important general fact that the "rebound" is not as marked in lower levels of the stem as it is in the apex (*cf.* figs. 1 and 2).

At room temperature *A* is slightly electropositive to *C;* but lowering the temperature lowers the P.D. in the region *C*, with the result that the segment *AC* attains a large polarity followed by a marked "rebound" of the E.M.F. in the region *C* which temporarily *inverts* the polarity of *AC*. The existence of the "rebound" is unmistakable but smaller than that in the apex as shown in figure 1.

At room temperature *DE* has little or no polarity, but cooling causes the appearance of an inverted polarity with return to a normal polarity associated with a smaller but distinct "rebound" which tends to persist. The change in segment *FD* follows closely the change in *DE*. This merely shows that it is the E.M.F. in the region *D* which is affected and not that in regions *E* or *F*.

In the upper set of curves, *CD* shows that the P.D. between the regions *D* and *C* becomes distinctly less at low temperature. But the difference in the effect of temperature upon the apical region *C* and the more basal region *D* is relatively small in comparison with the difference in a more apical region, such as in figure 1, curve *AB*. The evidence in the curves for a variation of E.M.F.'s in the segments *AB* and *EF* is apparently indefinite and needs further detailed investigation.

The question whether or not lowering of the temperature of the segment *DC* lowers the E.M.F. between *F* and *A* is of special interest when considering the validity of the principle of summation of E.M.F.'s in polar tissues. Since the drop in E.M.F. in *CD* is relatively small, the relatively small change in the curve *AF* may have some significance. From experiments carried out in a different manner, it appears that the procedure as indicated in figure 2 is not well adapted to bring out the facts on this point, especially when changes in temperature on segments from the more basal levels of the stem are observed. This question will be touched upon again in the following paper. (See also MARSH **8**.)

Significance of results

From the facts presented in previous papers (LUND **4**, **5**), it appears clear that the E.M.F.'s which constitute the characteristic polar properties of the Douglas fir have their origin in the living cells. What is observed

when measuring the external longitudinal electric polarity, as in these experiments, is the resultant of a rather complex system of E.M.F.'s with two main groups of structural origins, namely, living wood and living cortex, both of which are derived from the bipolar cambium. It has also been fully shown that the fundamental electrical characteristics of a polar group of cells are directly referable to the same characteristics in a single cell. The facts are summed up in the statement that the electric polarity of the organ is the algebraic sum of the electric polarities of its cells.

Now the only way, from the view-point of electrochemistry, in which it is at present possible to think of the electric polarity of a cell, is that this E.M.F. is the algebraic sum of elementary regional or phase boundary (loci) potentials having an aggregate polar orientation. The simplest expression which defines the conditions of such a system is that for two oppositely oriented potential differences.

If for the moment we assume that this system is an oxidation reduction system in the state of flux equilibrium, and for the sake of simplicity consider only two of the electromotively active species of reactants in the two loci in the cell, then for the polarity potential of the cell we may write

$$E_p = \frac{RT}{2F} \ln \left(\frac{[A]_b \, [AH_2]_a}{[AH_2]_b \, [A]_a} \right), \qquad (1).$$

In order to make this expression apply to a large number of loci, we should in the ideal case merely have to sum all the elementary E.M.F.'s in a complex system in order to obtain an expression representing the total E.M.F. (*cf.* LUND **6**, p. 245).

With this conception in mind, the important question is, how would temperature be expected to affect the E.M.F. of such a polar system? If we assume for the moment that the above equation represents the E.M.F. of a system in *static* equilibrium, such as a simple concentration potential, then it is evident that the E.M.F. would be directly proportional to the absolute temperature, for the ratio of concentrations of A and AH_2 in any of the loci would not be changed in such a state of perfect reversibility. The observed effects of temperature on such a system are of course relatively small and similar to what BERNSTEIN found for the resting and injury potential (*cf.* metastable state, above) in a muscle.

Now if we consider the total E.M.F. of two oppositely oriented homologous cells, one located in the apical region *A* and the other in the more basal region *B* in figure 1, then the expression for the total E.M.F. becomes

$$\Sigma E_p = \frac{RT}{2F} \left[\ln \left(\frac{[A]_b \, [AH_2]_a}{[AH_2]_b \, [A]_a} \right)_1 - \ln \left(\frac{[A]_b \, [AH_2]_a}{[AH_2]_b \, [A]_a} \right) \right], \qquad (2).$$

The subscripts 1 and 2 denote respectively apical and basal cells. Again

it is obvious that the change in total E.M.F. with *equal* change in temperature around the apical and basal cells is small and directly proportional to the absolute temperature. Lowering of the temperature of such a system obviously could not produce as great an effect as that represented by the drop in curve *AB* in figure 1. It could not at all account for the "rebound" phenomenon shown by curve *AB* when the system is returned to room temperature.

These facts, together with the further fact that the system is an open one which generates energy, dispose completely of all attempts to view the equilibria of these *continuous* bioelectric currents associated with life activities in terms of simple concentration potentials of systems in static, metastable, or dynamic equilibrium.

The only obvious alternative way in which these effects of temperature on electric polarity can be explained is that change in temperature changes in a characteristic manner the relative flux concentrations of the corresponding electromotively active substances in the apical and basal regions *A* and *B*. Since the electrochemical system is in a state of flux equilibrium, the same change in temperature must affect *unequally* the velocities of the individual links in the catenary series of reactions which are in the state of flux equilibrium.

In short, here is an indirect method of showing that the temperature coefficients of the velocities of the different links in the chain of reactions which constitute the mechanism for producing electrical energy by the cell are different. Raising or lowering the temperature results in increasing or decreasing selectively the concentration of certain electromotively active components at the seat of origin of E.M.F., thus changing the ratio of the concentrations in equation (2) above. When the system is returned to the former temperature, then the concentrations of the links in the catenary system return approximately to their former relative values. This is the explanation of the "rebound" phenomena in the curves. The magnitude of the "rebound" is determined by the magnitude of the unequal changes of the concentrations. These facts, especially when taken in conjunction with (a) the "rebound" effects on the E.M.F. in the frog's skin caused by changing the oxygen concentration; (b) the "rebound" effects produced in the output of CO_2 by *Planaria* after a period in the absence of oxygen; and (c) the unequal effects of equal change in oxygen concentration on the velocity of oxidation in apical and basal ends of the *Obelia* stem (LUND **3, 7, 6**), constitute a conclusive proof that the electrochemical mechanism which produces continuous bioelectric currents in the living cell is in a state of flux equilibrium.

The question remains, is this system in the Douglas fir an oxidation reduction system which is linked quantitatively with cell oxidation as has

been shown in the case of *Obelia*, roots, and the frog's skin? In the absence of experimental facts an answer to this question is postponed.

Another interesting question is, how are the directions and magnitudes (patterns) of the internal correlation currents in the wood and cortex modified when the polarities are changed by temperature? A clearer understanding of how change in temperature can be used to modify this pattern of distribution of correlation potentials is of obvious importance in the practical solution of the problem of how such correlation processes as inhibition and dominance of growing points operate in the developing organism. In a following paper further light will be thrown on some of these questions.

Summary

1. Additional evidence is presented to show that temperature affects the E.M.F. of a system in flux equilibrium by changing the relative concentrations of the electromotively active substances which represent the links in the catenary series of reactions in flux equilibrium. The other effect of temperature on such a system is small and proportional to the absolute temperature, but is purely imaginary since the system is not reversible and therefore is not in thermodynamic equilibrium.

2. By lowering the temperature equally around an apical segment of the main axis or lateral branch of the Douglas fir, the electric polarity of this segment is diminished. This is due to unequal effect of equal change in temperature on apical and basal regions of the piece, and is related to the difference in the amount of change in the ratios of concentrations of the electromotively active substances in apical and basal levels of the piece.

3. Return of the previously cooled segment to the original temperature results temporarily in what has been called a rebound of the E.M.F. This rebound is relatively greater in apical than in basal regions of the stem. This phenomenon is a logical consequence of the nature of the effect of temperature on the flux equilibria.

4. The electric polarity of the apex of the Douglas fir may be increased, decreased, reversed, or made equal to zero by means of temperature. By inference, the internal correlation currents are therefore changed in a corresponding manner. The possible significance of these phenomena for regulation in growth and other processes is pointed out.

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FLOWER PRODUCTION FROM GLADIOLUS CORMS HARVESTED AT DIFFERENT STAGES OF RIPENING[1]

B. E. Gilbert and F. R. Pember

Differences of opinion have been found to exist among commercial gladiolus growers as to the proper stage of maturity at which to harvest gladiolus corms to insure later maximum flower production. Some growers are very careful to allow the tops to dry out before digging the corms; others dig immediately after flower production is past and allow the plants to dry out exposed to the sun and air before cutting off the tops; while others claim that neither of these precautions is necessary and that as good flower production may be secured by digging immediately after the flowering period and severing the green tops from the corms at once.

An experiment was carried on during 1930 and 1931 at the Rhode Island Agricultural Experiment Station to determine the effect on yield of blossoms of plants grown from corms harvested according to these methods.

Cultural methods

During the spring months of 1930, plants of the Alice Tiplady and Maiden's Blush varieties were grown in the greenhouse in fertile soil to which optimum nutrients were added. Both varieties flowered during the months of May and June. The Alice Tiplady plants were harvested on three different dates, with the following treatments:

1. July 14: 25 plants harvested and taken for chemical analysis.
   20 plants harvested and topped at once.
   20 plants harvested and allowed to dry in the sun with the corms covered, and topped September 11.
2. August 7: 25 plants harvested and taken for chemical analysis.
   20 plants harvested and topped at once.
   20 plants harvested and allowed to dry in the sun with the corms covered, and topped September 11.
3. September 11: 100 plants dried out in the bed, harvested, and topped.
   25 plants taken for chemical analysis.

Two harvests were made with the Maiden's Blush plants, and treatments were as follows:

1. July 17: 400 plants were harvested and of these
   100 were topped at once,
   25 were taken for chemical analysis,
   275 were stored exposed to sun and air with corms covered.

[1] Contribution no. 417 of the Rhode Island Agricultural Experiment Station.

2. August 7: 25 of the preceding 275 taken for chemical analysis.
3. September 11: Remainder topped.

The corms which had been harvested as indicated were stored in a cool dry cellar throughout the summer and were planted in the greenhouse on February 19, 1931, in fertile soil to which optimum nutrients had been added. The plants from these corms bloomed during May and June, thus giving data showing the effect of harvesting methods on growth and yield.

Methods of chemical analysis

Essentially the same procedure as that used by POTTER and PHILLIPS (**1**) with apple spurs was followed in preserving, extracting, and fractionating, with the following exceptions:

PRESERVING THE SAMPLE.—Immediately after harvesting, 25 plants were brought to the laboratory. The corms were severed from the tops at the base of the stalk. The tops and corms were quartered and one-quarter of each was cut up into fine pieces and then ground through a Nixtamal mill. The ground material was weighed. Two samples of 10 gm. each were weighed into tared moisture dishes and the moisture determined. Two samples of 100 gm. each were weighed into tared Erlenmeyer flasks, 0.25 gm. $CaCO_3$ and 150 cc. hot 95 per cent. alcohol added, and the flask heated to gentle boiling for 30 minutes.

DETERMINATION OF CARBOHYDRATE FRACTIONS.—Sugars were determined by the use of the SHAFFER-HARTMAN iodimetric method (**2**) for the estimation of cuprous oxide.

A major exception from the methods of POTTER and PHILLIPS was found to be necessary in the manner of estimating acid-hydrolyzable polysaccharides. It was found impossible to secure consistent results from the acid hydrolysis of the residue from the starch determinations, and so the acid-hydrolyzable polysaccharides were determined on 1 gm. of the dried residue. Thus this fraction in this experiment contains starch and is essentially different from that of POTTER and PHILLIPS.

Yield data

In table I data are given showing the yield of blooms and corms from plants grown from corms which had been harvested in various ways.

Alice Tiplady plants were harvested on July 14, August 7, and September 11, 1930, and immediately topped. Plants grown from these corms in 1931 gave no indications of significant differences in yields of corms or flower stalks, considering a 20 per cent. difference to be the lower limit of a significant yield variation. There was, however, a consistent increase in the average length of flower stalk and number of florets as the 1930 corms were allowed to remain longer in the bed.

TABLE I

YIELD OF GLADIOLUS PLANTS GROWN FROM CORMS MATURED BY DIFFERENT METHODS

VARIETY	HARVEST 1930	TOPPED 1930	BLOOMS, 1931			CORMS, 1931	
			No.*	AVERAGE LENGTH	AVERAGE NO. PER PLANT	No.*	AVERAGE WEIGHT PER CORM
				inches			*gm.*
Alice Tiplady	July 14	July 14	100	28.5	8.0	105	12.8
	Aug. 7	Aug. 7	100	29.0	8.5	95	20.6
	Sept. 11	Sept. 11	111	32.0	9.0	105	16.9
	July 14	Nov. 11	115	28.0	8.0	130	16.1
	Aug. 7	Nov. 11	95	26.5	7.5	110	17.5
Maiden's Blush	July 17	July 17	101	32.5	11.5	100	25.0
	July 17	Nov. 11	102	33.5	11.0	112	25.8

* Based on 100 plants.

Corms from Alice Tiplady plants harvested July 14 and August 7, 1930, and allowed to dry out with the tops exposed to the sun and air, produced plants in 1931 which yielded essentially the same in blooms and corms. There were no appreciable differences in the average lengths of flower stalks or average number of florets.

The Maiden's Blush corms which were harvested July 17, 1930, and immediately topped, when compared with similar corms harvested on the same date and the tops allowed to dry in the sun, gave plants in 1931 which were essentially the same in all yield data.

Chemical determinations

The results of the determinations of the carbohydrate and nitrogen fractions are given in tables II to V. Attention is drawn particularly to tables III and V, where the results are expressed as actual grams of the analytical fractions in 25 plants of the two varieties of gladiolus.

As noted previously, the Alice Tiplady plants (table III) which were analyzed were harvested July 15, August 3, and September 11, 1930. The plants harvested September 11 might be considered to have been metabolizing actively until the middle of August at the least. After that time the tops gradually dried out, until when harvested they were air-dry. Thus the acid-hydrolyzable polysaccharides and the alcohol-insoluble nitrogen accumulated in the tops and corms. Apparently the greatest increase must have been in cellulose, although this cannot be demonstrated as that fraction

TABLE II

ANALYTICAL FRACTIONS EXPRESSED AS PERCENTAGES OF DRY MATTER (ALICE TIPLADY)

DATE OF HARVEST	TOPS			CORMS		
	JULY 15	AUG. 3	SEPT. 11	JULY 15	AUG. 3	SEPT. 11
Dry matter*	20.96	24.58	93.10	24.67	29.65	31.65
Reducing sugar	5.30	4.13	0.74	2.89	1.79	0.82
Sucrose	Trace	1.38	Trace	7.42	7.47	4.49
Starch	Trace	Trace	Trace	46.17	47.83	47.39
Acid-hydrolyzable polysaccharides	1.48	1.57	0.96	37.19	40.24	38.55
Alcohol-soluble nitrogen	0.27	0.15	Trace	0.71	0.63	0.81
Alcohol-insoluble nitrogen	1.28	0.95	0.74	1.15	1.34	1.85
Total nitrogen	1.55	1.10	0.74	1.86	1.97	2.66

* In the undried material.

TABLE III

ACTUAL GRAMS OF ANALYTICAL FRACTIONS IN 25 PLANTS (ALICE TIPLADY)

DATE OF HARVEST	CORMS			TOPS		
	JULY 15	AUG. 3	SEPT. 11	JULY 15	AUG. 3	SEPT. 11
Dry matter	209.60	444.06	837.90	394.72	405.61	443.10
Reducing sugars	11.12	18.27	6.21	11.41	7.27	3.66
Sucrose	Trace	6.09	Trace	29.31	30.30	19.89
Starch	Trace	Trace	Trace	182.24	194.00	209.98
Acid-hydrolyzable polysaccharides	3.10	6.97	8.04	146.80	163.22	170.81
Alcohol-soluble nitrogen	0.56	0.67	Trace	2.79	2.56	3.59
Alcohol-insoluble nitrogen	2.68	4.22	6.20	4.54	5.43	8.20
Total nitrogen	3.24	4.89	8.20	7.33	7.99	11.79

was not included in the analysis. In the corms the starch fraction increased with maturity, partly at the expense of the soluble sugars, which decreased. The total nitrogen of the corms may have increased owing to the translocation of soluble nitrogen from the tops. In both carbohydrate and nitrogen fraction the movement was toward the accumulation of storage forms in the corms. This is as would be expected with storage in corm formation.

Table V gives the results of the analysis of two lots of 25 Maiden's Blush plants which differed only in the treatment after harvesting. The tops of the plants which were allowed to dry out in the sun apparently were sub-

TABLE IV

ANALYTICAL FRACTIONS EXPRESSED AS PERCENTAGE OF DRY MATTER (MAIDEN'S BLUSH)

DATE OF HARVEST	TOPS		CORMS	
	JULY 17	AUG. 3	JULY 17	AUG. 3
Dry matter*	26.39	89.70	30.52	33.70
Reducing sugar	5.49	0.15	1.03	0.69
Sucrose	0.61	Trace	5.86	4.90
Starch	Trace	Trace	53.62	44.23
Acid-hydrolyzable polysaccharides	2.15	2.08	35.52	41.52
Alcohol-soluble nitrogen	0.18	0.26	0.56	0.58
Alcohol-insoluble nitrogen	0.62	0.50	2.10	1.13
Total nitrogen	0.80	0.76	2.66	1.71

* In the undried material.

TABLE V

ACTUAL GRAMS OF ANALYTICAL FRACTIONS IN 25 PLANTS (MAIDEN'S BLUSH)

DATE OF HARVEST	TOPS		CORMS	
	JULY 17	AUG. 7	JULY 17	AUG. 7
Dry matter	263.90	259.00	488.32	539.20
Reducing sugars	14.50	4.59	5.02	3.71
Sucrose	1.60	Trace	28.60	26.45
Starch	Trace	Trace	261.84	238.49
Acid-hydrolyzable polysaccharides	5.67	0.54	173.45	223.87
Alcohol-soluble nitrogen	0.49	0.79	2.73	3.15
Alcohol-insoluble nitrogen	1.64	1.29	10.25	6.09
Total nitrogen	2.13	2.08	12.98	9.24

ject to translocation from tops to corms, as all the fractions (with the exception of the soluble nitrogen) decreased materially in actual amounts, while there was an increase in the acid-hydrolyzable polysaccharides in the corms. It is suggested that while the decrease in the reducing sugars and sucrose in the corms may have been due partly to respiration, the greater part of the loss in these fractions was due to condensation to polysaccharides. The total nitrogen of the corms decreased, owing to a marked decrease in the insoluble nitrogen.

Discussion

In general, the changes noted by the analysis of carbohydrate and nitrogen fractions are in line with the theory of condensation of simple compounds to complex storage forms which normally takes place during ripening. Theoretically, therefore, the additional amounts of condensed compounds in the corms which had been allowed to mature before being

dug, or having the tops severed, might be expected to give the better plants and the better yield. Referring to table I, however, there seems to have been but slight differences between the yields of the 1931 crop despite the harvesting treatment of the 1930 plants. No appreciable size differences were noted in connection with the Alice Tiplady corms harvested at different dates. This fact is also in agreement with the data in table I. From other unpublished data it has been determined that size of corm affects flower production. Where such differences have been noted, however, the size differences have been many times greater than any noted in this experiment. This leads to the conclusion that differences such as were found to be present in amounts of storage chemical compounds, were perhaps not great enough to influence the succeeding generation of gladiolus. In other words, usually a gladiolus corm may be considered to have huge reserves of energy, only a portion of which is needed to establish and maintain the new plant.

Conclusions

From data obtained by growing gladiolus in the greenhouse from corms, some of which were harvested at different stages of maturity, some of which had the tops removed immediately after harvest, and some of which were allowed to dry out before topping, it is concluded that there is little to gain by any one of these procedures over the others. Differences in flower and corm yields were not significant. There seemed to be a slight benefit in length of flower stalk and number of florets accompanying greater maturity; for example, the Alice Tiplady corms which were allowed to mature in soil gave longer stalks and more florets per stalk.

Chemical analyses gave data showing accumulation of condensed forms of carbohydrates and nitrogen compounds stored particularly in the corms. These additional amounts, however, were apparently not great enough to influence the yield of the succeeding generation of gladiolus.

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FIELD METHOD FOR ESTIMATING NITRATE, PHOSPHATE, AND POTASSIUM IN PLANTS[1]

E. M. EMMERT

Introduction

Work on tomato and lettuce plants (**2**) indicates that the percentage of a plant-food element in the mature conducting tissue of a plant is a better index of the adequacy of the soil in supplying this element to the plant than is the percentage of the element found in the soil. Therefore quick tests for approximating the percentages of nitrate, phosphate, and potassium in mature plant tissue, in the field, should have practical value in supplying information to guide the application of fertilizers, either to the growing crop or for the benefit of a crop to follow. The procedures described in this paper, which are adaptations of good laboratory procedures, seem to be satisfactory for the purpose indicated.

The field pack should be as follows:

FOR PREPARING THE PLANT EXTRACT.—A small hand balance and 5 gm. weight; a 10 cc. graduate; two small mortars and pestles; 11 cm. no. 1 Whatman filter-papers; test-tubes ($3\frac{1}{2}'' \times \frac{1}{2}''$ is a convenient size); a test-tube rack; a dipper to measure 0.5 gm. of charcoal; a 1 cc. pipette graduated in tenths of a cc.; glacial acetic acid; phosphate-free charcoal (**3**); tap water (distilled water is best if available). Besides these, calcium hydroxide and a 10 per cent. solution of copper sulphate (**4**) may be needed for some tissues.

FOR THE NITRATE TEST.—A 25 cc. evaporating dish; an alcohol lamp; a pair of tongs; a 25 cc. graduate; test-tubes ($3\frac{1}{2}'' \times \frac{1}{2}''$); phenoldisulphonic acid (**1**) (solution An); clear 30 per cent. sodium hydroxide (solution Bn); a set of color standards, made as directed under the nitrate test.

FOR THE PHOSPHATE TEST.—A 1 cc. pipette; a 2.5 per cent. solution of ammonium molybdate in 5 N sulphuric acid (solution Ap); aminonaphthol sulphonic acid (solution Bp) (**6**); a set of color standards, made as directed under the phosphate test.

FOR THE POTASSIUM TEST.—A 25 cc. evaporating dish; sodium cobaltinitrite solution (**5**) (solution Ak); a solution made by dissolving 27 gm. of sodium nitrite in distilled water and making up to 100 cc. (solution Bk); a set of color standards, made as directed under the potassium test.

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

Preparation of plant extract

Portions of the lower mature main stem or of lower mature petioles or branches as near the main stem as possible should be used. Immediately weigh 5 gm. of the fresh tissue as nearly whole as possible and cut into small pieces after weighing. Grind in a mortar with 0.5 gm. of phosphate-free charcoal (use more charcoal if needed to assure a clear solution). Add 10 cc. of 2 per cent. by volume acetic acid as the grinding is being done. Immediately mix thoroughly and filter through a dry filter into a test-tube. Each 0.2 cc. of this solution represents 0.1 gm. of sample. If the nitrate test cannot be made immediately, take the aliquot for the nitrate test and add an equal volume of 3 per cent. NaOH before allowing the extract to stand.

The extract usually is clear if stem or petiole tissue is used. Extract from leaf tissue may or may not be clear and the protoplasm of leaf cells may retain all the water, making it necessary to use more 2 per cent. acetic acid.

The succulence of the tissue varies the amount of extract secured, making it necessary to use the same weight of sample and the same volume of 2 per cent. acetic acid in making comparisons. The approximate parts per million of the nutrient can be found by making moisture determinations and allowing for the moisture of the sample. Comparisons can be made without doing this, since the amount of moisture in the same kind of tissue does not fluctuate enough to be significant in approximate analyses.

Nitrate test

This test should be made at once, to avoid enzymic reduction which will occur if the extract is allowed to stand. Put enough of the extract (usually 0.2–1.0 cc.) to give a good yellow nitrate color into a 25-cc. evaporating dish, add an equal volume of 3 per cent. NaOH, and evaporate to dryness without spattering or burning, over an alcohol lamp. Add 1 cc. of phenol-disulphonic acid (An) and cover all the residue well by rotating the dish. Let stand about 5 minutes and add about 10 cc. of water. Add clear 30 per cent. NaOH (Bn) slowly until a yellow color persists. Make up to 20 cc. and compare with the color standards for nitrate nitrogen.

PERMANENT COLOR STANDARDS FOR NITRATE NITROGEN.—Dissolve 0.3609 gm. of KNO_3 in distilled water and make up to 1000 cc. One cc. of this solution contains 0.05 mg. of nitrate nitrogen, and 0.1 cc. corresponds to 50 ppm. of nitrate nitrogen in the plant tissue, if 0.1 gm. of plant tissue is represented. If the extract from 0.2 gm. of tissue has been used, the color will represent 25 ppm.; if 0.4 gm. of tissue was required, the color represents 12.5 ppm., etc.

Prepare a series of standards as indicated in table I.

TABLE I

STANDARD NITRATE SOLUTION	NITRATE NITROGEN REPRESENTED IN GREEN TISSUE WHEN 0.1 GM. IS USED IN NITRATE TEST	
cc.	*ppm.*	
0.1	50	(Color varies from faint
0.2	100	yellow at 50 ppm. to
0.4	200	very strong yellow at
0.8	400	1000 ppm.)
1.2	600	
1.6	800	
2.0	1000	

These standards will last indefinitely if kept in glass-stoppered tubes. They will keep in the test-tubes if stoppered with rubber stoppers and care is taken that the solution does not come into contact with the stoppers.

COLOR INTERFERENCE IN NITRATE TEST.—If the nitrate test cannot be read accurately because of interfering substances, grind 5 gm. of tissue with about 0.5 gm. of calcium hydroxide, add 10 cc. of 2 per cent. copper sulphate solution (2 cc. of 10 per cent. diluted to 10 cc. with water), mix well, allow to stand about 5 minutes, and filter (**4**). (The extract prepared in this way is not suitable for estimating phosphate phosphorus.) The $Cu(OH)_2$ usually clarifies the solution satisfactorily.

Phosphate test

Put sufficient plant extract (usually 0.2–2 cc.) to give a good color into a 10-cc. graduate. Make up to about 7 cc. with water. Add 1 cc. of molybdate solution (Ap) and 0.5 cc. of aminonaphthol sulphonic acid (Bp). Make up to 10 cc., mix, and allow to stand 10 minutes before comparing with the color standards for phosphorus.

TEMPORARY PHOSPHATE PHOSPHORUS COLOR STANDARDS.—Dissolve 0.1097 gm. of KH_2PO_4 in distilled water and make up to 2500 cc. One cc. of this solution contains 0.01 mg. of phosphate phosphorus and 0.1 cc. represents 10 ppm. of phosphate phosphorus in the plant tissue, if 0.1 gm. of plant tissue is represented. If 0.2 gm. of tissue has been used the color represents 5 ppm., etc.

Prepare a series of standards as indicated in table II. These standards must be made up at the same time as the unknown to secure proper comparison. The color deepens with standing.

PERMANENT PHOSPHATE PHOSPHORUS COLOR STANDARDS.—Measure out two 5 cc. portions of the phosphate standard solution prepared for the temporary standards. Develop the blue color as directed for the plant extract. Allow to stand 72 hours. Prepare a set of temporary standards. Dupli-

TABLE II

STANDARD PHOSPHATE SOLUTION	PHOSPHORUS IN GREEN TISSUE WHEN 0.1 GM. IS USED IN TEST	
cc.	*ppm.*	
0.1	10	(Color varies from very
0.2	20	faint blue at 10 ppm.
0.5	50	to very dark blue at
1.0	100	440 ppm.)
2.0	200	
3.0	300	
4.0	400	

cate the colors developed by the temporary standards in 10 minutes by diluting appropriate amounts of the dark blue solution which has stood 72 hours with a clear solution made by adding 10 cc. of acid ammonium molybdate (Ap) and 4 cc. of aminonaphthol sulphonic acid (Bp) to 70 cc. of distilled water and making up to 100 cc. If the color is too light add a little more of the dark blue solution, or if it is too dark dilute with the clear acid molybdate solution until a set of standards made from the blue solution which has stood 72 hours exactly duplicates the temporary set. The permanent set usually will keep for several days, but for accurate work it is best to make up fresh standards at the time the tests are made.

Potassium test

Put sufficient plant extract (usually 0.5–2 cc.) to cause an appreciable reduction of color of the potassium reagent into a 25-cc. evaporating dish and evaporate almost to dryness, without spattering or burning. Add 0.3 cc. of sodium cobaltinitrite (Ak), cover all the residue by rotating the dish, and allow to stand about 5 minutes. Make to 10 cc., in a 10-cc. graduate, with sodium nitrite solution (Bk). After the precipitate settles, compare with the color standards for potassium. The comparison may be made immediately by filtering off the precipitate on a dry filter. The lighter the color the greater the amount of potassium. If the solution is nearly colorless, another test should be made, using less plant extract.

TEMPORARY POTASSIUM STANDARDS.—Dissolve 1.2929 gm. of KNO_3 in distilled water and make up to 250 cc. One cc. of solution contains 2.0 mg. of potassium and 0.3 cc. represents 600 ppm. of potassium in the plant tissue, if 1.0 gm. of plant tissue is represented. If 2.0 gm. of tissue has been used the color represents 300 ppm., etc.

Prepare a series of standards as indicated in table II. These standards are good only for the day they are made; the color fades slowly on standing over night.

TABLE III

STANDARD POTASSIUM SOLUTION	POTASSIUM REPRESENTED IN GREEN TISSUE WHEN 1.0 GM. IS USED IN TEST	
cc.	*ppm.*	
0.00	0	(Color varies from dark
0.30	600	brown color at 0 ppm.
0.60	1200	to faint yellowish
0.90	1800	brown at 3000 ppm.)
1.20	2400	
1.35	2700	
1.50	3000	

PERMANENT POTASSIUM STANDARDS.—When ferric chloride is dissolved in concentrated hydrochloric acid, a permanent color is produced which is almost the same as the sodium cobaltinitrite color. Make a strong solution of $FeCl_3$ in hydrochloric acid. Duplicate the color of the temporary potassium standards by diluting appropriate amounts of the $FeCl_3$ solution with hydrochloric acid. These solutions will keep indefinitely.

TABLE IV

COMPARISON OF LABORATORY AND FIELD TESTS FOR NITRATE AND PHOSPHATE, MADE ON THE SAME MATURE TOMATO PETIOLES (PPM. OF GREEN PLANT TISSUE)

PLANT	TREATMENT	NITRATE N		PHOSPHATE P		NOTES ON APPEARANCE OF PLANT
		LABORATORY	FIELD	LABORATORY	FIELD	
		ppm.	*ppm.*	*ppm.*	*ppm.*	
1	Check; a rather poor red soil	980	1000	Trace	Trace	Fairly green and large; fruit set poor
2	400 lb. of sodium nitrate per acre	1666	1600	266	200	Green, large plants; fruit set fair
3	20 tons manure and 1 ton $Ca(OH)_2$ per acre	1500	1300	300	250	Quite vegetative and large; fruit set fair
4	400 lb. of sodium nitrate per acre	1666	1600	266	200	Same as plant no. 2
5	4 tons of straw per acre	Trace	None	660	500	Stunted, yellow plants; fruit set good
6	40 tons of manure, 1 ton $Ca(OH)_2$, 0.5 ton Na_2CO_3	1666	1500	600	400	Very large; vegetative plants with good set of large fruits

Results

Comparison of field and laboratory tests

Table IV shows quantitative results obtained by the laboratory procedure (**4, 5**) and the amounts estimated by the field tests in samples from the same plants. The two sets of determinations agree well, considering that the field test is approximate. The nitrate determinations check quite closely, while the laboratory values for phosphate are slightly higher than those obtained by the field test.

Table IV also shows a good correlation between fruit set and phosphate phosphorus content, as well as between vegetation and nitrate nitrogen content. The appearance of the plant cannot always be relied upon, however, since some disease or nutritional factors other than lack of nitrate may cause a yellowish color. Many other factors besides lack of phosphorus may cause a poor set.

Results of field tests on different plants

In order to determine whether the tests would work on different plants, determinations were made on plants of several kinds. The results are presented in table V. In a few of these, interference by carbohydrate was

TABLE V

Results of field tests (ppm. of green tissue)

Plant	N	P	K
	ppm.	*ppm.*	*ppm.*
Peas (stunted), stems	Trace	700	1000
Peas (vigorous), stems	800	300	2500
Rhubarb (vigorous), petiole	150	800	500
Coleus (vigorous), stem	2000	350	.....
Onion (vigorous), stem	250	300	700
Asparagus (stem tissues of tips)	Trace	600	900
Peach, petioles	600	1000	1500
Raspberry (vigorous), petioles	300	5	800
Raspberry (mosaic yellow), petioles	50	10	400
Potted tomato plant (root-bound and yellow)	50	30	600
Potted tomato plant (green and stocky)	400	15	400
Young corn plants (vigorous)	700	10	1800
Young peach fruits	100	900	600

experienced in making the nitrate test on the charcoal extract. When $Ca(OH)_2$ and copper sulphate were used as the clarifying agents, the difficulty was overcome. The kinds of tissue in which this occurred were fresh asparagus tips, green onions, and small peach fruits.

Interpretation of field tests

As yet, not enough tests have been made to determine the exact concentrations of nutrients which should be maintained in the various plants to obtain optimum yields. However, the concentration found in vigorous, high-yielding plants should be a guide to the amount of nutrient which should be present. For instance, table IV shows that the vigorous tomato plants possessing a good set of fruit contained about 1600 ppm. of nitrate nitrogen, 600 ppm. of phosphate phosphorus, and 3800 ppm. of potassium. The nitrated plants contained 1600 ppm. of nitrate nitrogen and looked healthy, yet the fruit set was not good. The tests revealed that only 200 ppm. of phosphate phosphorus was present. The inference is that it would pay to add available phosphorus. Field tests can thus be used to reveal nutrient deficiencies and to form a basis for intelligent fertilizer application to supply the specific need of a particular crop.

Field tests on the plant indicate what soil nutrients are available to the plant, and enable the determination of the optimum ratios and concentrations of nitrate, phosphate, and potassium for each kind of plant. The interference experienced in soil tests with certain soils is eliminated. The tests may be performed rapidly in the field, dispensing with the necessity of a laboratory and hours of laboratory routine.

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SEASONAL CHANGES IN THE COMPOSITION OF THE INSOLUBLE NITROGEN FRACTION IN THE CURRENT YEAR'S SHOOTS OF BARTLETT PEAR

A. S. Mulay

(WITH TWO FIGURES)

In a previous paper (**1**), it was shown that there were considerable variations in the quantity of insoluble nitrogen, both in bark and wood, during the growth cycle of pear shoots. It was also shown in another paper (**2**) that seasonal variations in the water-soluble nitrogen fraction in pear shoots are not only quantitative but qualitative also. It was of interest to find how the insoluble nitrogen fraction, representing mainly cytoplasmic proteins, behaved in this respect. As a convenient way of partially answering this question, a Van Slyke fractionation of the insoluble nitrogen was undertaken, although it was apparent that the constancy of results by this method would be no proof of the constant composition of this fraction.

Experimentation

Current year's shoots were separated into bark and wood, dried at 50° C., and ground to 100 mesh. This powder was shaken with water at room temperature for two hours, and the residue separated from the soluble extract by centrifuging and washing. This residue was dried at 50° C. and powdered. The amount of total nitrogen in this residue is called insoluble nitrogen. The material used and the procedure followed are fully described in the paper (**1**) on the seasonal variations in soluble and insoluble nitrogen. A 25-gram portion of this residue was extracted with benzene and alcohol in the order named and the nitrogen determined in these extracts. The extracted sample was gently boiled with 20 per cent. hydrochloric acid for 48 hours under a reflux condenser. The hydrolysate was filtered under suction on hardened filter-paper and the residue washed with hot water until free from chlorides. The filtrate and the washings were evaporated nearly to dryness under reduced pressure. This concentrate was taken up with hot water, made up to 500 cc., and total nitrogen determined on an aliquot. The remainder was carried through the amide and other determinations as described elsewhere (**2**). Tables I and II summarize the results obtained.

Discussion

The insoluble nitrogen of the bark (fig. 1) contains 40–50 per cent. amino nitrogen, nearly 20 per cent. basic nitrogen, 10–15 per cent. melanin nitrogen, 7–9 per cent. amide nitrogen, 4–6 per cent. humin nitrogen, 5–10 per cent. rest nitrogen, 2 per cent. alcohol-soluble nitrogen, and about 0.1

TABLE I

INSOLUBLE NITROGEN FRACTIONATION
AS PERCENTAGE OF INSOLUBLE NITROGEN
BARK

DATE COLLECTED	BENZENE EXTRACT	ALCOHOL EXTRACT	MELANIN	NITROGEN IN HYDROLYSATE	AMIDE	HUMIN	BASIC	AMINO	REST
Sept. 16	0.1	2.0	16.0	82.0	8.1	4.4	17.4	44.5	7.8
Oct. 21	0.1	1.7	16.3	82.2	8.1	3.6	17.2	43.4	10.3
Dec. 7	.....	2.1	14.3	83.6	7.2	5.1	20.9	40.9	9.6
Jan. 15	.....	2.2	9.7	88.1	7.9	6.3	23.2	46.8	4.2
Feb. 11	0.2	2.2	9.7	89.2	8.4	5.6	22.7	47.5	4.2
Mar. 18	.....	2.0	12.9	85.3	8.2	4.3	24.6	44.6	3.6
Apr. 18	0.3	2.4	10.2	87.5	9.2	5.1	20.5	46.8	7.3
May 13	.....	2.2	10.9	86.8	9.1	6.5	19.9	44.9	6.5
June 24	0.3	2.0	5.3	92.8	8.7	4.0	22.9	51.4	5.9
July 15	0.2	2.0	11.6	86.5	8.4	8.6	19.3	49.0	1.1
Aug. 19	0.1	2.0	10.8	87.2	8.6	4.3	20.8	52.0	1.4
Sept. 19	0.2	2.0	12.1	80.5	7.8	7.7	18.1	49.3	5.6
Oct. 15	.....	2.0	11.1	87.1	8.2	6.4	20.0	46.7	5.7
Nov. 17	0.2	2.0	15.4	80.7	7.8	7.9	18.3	43.4	4.5
Jan. 18	0.2	2.1	9.6	87.5	8.2	5.6	19.6	45.1	9.0

TABLE II

INSOLUBLE NITROGEN FRACTIONATION
AS PERCENTAGE OF INSOLUBLE NITROGEN
WOOD

DATE COLLECTED	BENZENE EXTRACT	ALCOHOL EXTRACT	MELANIN	NITROGEN IN HYDROLYSATE	AMIDE	HUMIN	BASIC	AMINO	REST
Sept. 16	0.4	7.3	10.9	81.3	13.3	3.2	10.8	.....	.....
Oct. 21	0.3	8.5	11.9	79.2	10.7	4.0	8.3	40.5	15.7
Dec. 7	0.2	7.2	12.2	80.6	12.4	4.0	11.9	36.0	16.4
Jan. 15	0.2	6.9	12.7	82.6	10.8	4.9	11.9	43.0	12.0
Feb. 11	0.2	7.8	12.6	79.5	11.4	3.6	11.2	39.5	13.5
Mar. 18	0.2	7.2	12.9	80.0	12.0	4.9	12.2	39.6	11.5
Apr. 18	.....	7.0	14.0	78.0	11.1	0.2	13.3	41.4	13.0
May 13	.....	5.7	13.8	80.6	11.2	1.2	14.2	40.2	13.7
June 24	.....	6.4	12.2	81.4	10.6	4.8	14.6	42.1	9.0
July 15	.....	8.6	11.2	80.8	10.5	7.3	13.2	39.3	10.5
Aug. 19	.....	9.8	18.1	72.1	9.3	3.1	15.1	35.0	9.2
Sept. 19	.....	8.2	22.7	70.5	9.4	5.1	13.6	33.5	9.2
Oct. 15	0.2	11.7	15.6	73.0	8.7	1.5	16.8	32.7	13.2
Nov. 17	0.2	8.7	17.3	74.1	8.5	0.4	13.5	37.7	14.1
Jan. 18	0.2	6.0	14.9	79.0	8.9	2.8	13.0	34.5	20.0

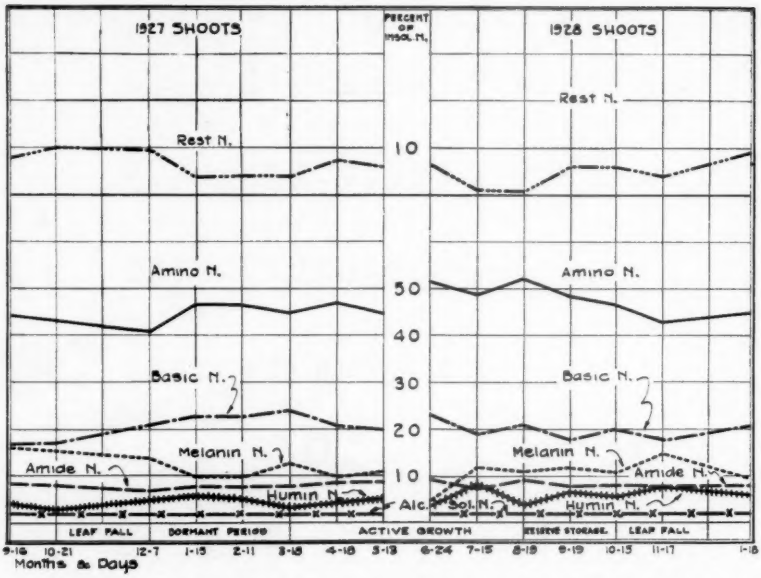

FIG. 1. Seasonal changes in composition of the insoluble nitrogen fraction in the bark of the current year's shoots of Bartlett pear.

per cent. benzene-soluble nitrogen. This composition seems to remain more or less constant throughout the year, except that at the beginning of the growing season there is a slight rise in amide and humin nitrogen at the cost of basic and rest nitrogen; and as growth proceeds there is a slight fall in amino nitrogen with a parallel rise in rest nitrogen.

The insoluble nitrogen of the wood is composed of about 40 per cent. amino nitrogen, 12 per cent. basic nitrogen, 14 per cent. melanin nitrogen, 10 per cent. amide nitrogen, 12 per cent. rest nitrogen, 4 per cent. humin nitrogen, 8 per cent. alcohol-soluble nitrogen, and 0.1 per cent. benzene-soluble nitrogen. The composition of the insoluble nitrogen of the wood, like that of the bark, is rather constant, except that from June to August, 1928, there was a marked fall in amino nitrogen and a rise in melanin nitrogen. There is a tendency for rest nitrogen to rise after active growth stops.

When compared with bark, wood seems to be especially rich in alcohol-soluble and rest nitrogen and poorer in basic nitrogen.

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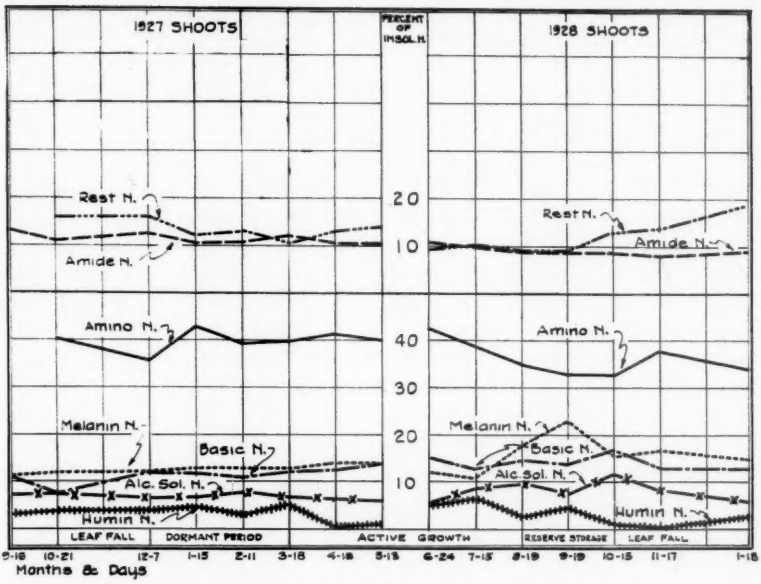

FIG. 2. Seasonal changes in composition of the insoluble nitrogen fraction in the wood of the current year's shoots of Bartlett pear.

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CHLOROSIS OF TREES[1]

EDMUND BURKE

(WITH ONE FIGURE)

Introduction

Chlorosis is a condition of the leaf in which chlorophyll fails to develop normally. BOURCART (**1**) states that this pathological condition is known as jaundice when caused by want or excess of water, and chlorosis when it is due to lack of iron or potash. An excess of carbonate of lime in the soil, insufficient nourishment, defective condition of the roots, a pathological condition of the whole tree, are some of the many causes which may produce chlorosis. When due to lack of iron, chlorosis has some analogy to the anemia of man.

Much has been published on the causes and treatment of chlorosis. No attempt is made to cite all the literature published, but a few citations are given to indicate the extent of its occurrence, and its treatment.

BOURCART (**1**) states that an excess of carbonate of lime is the predominating cause of chlorosis of trees with seed pips, such as the pear, and more especially of the vine. He recommends green vitriol for both trees and vineyards. KELLEY (**7**) found that an excess of manganese in the soil was responsible for the chlorotic condition of pineapples, as well as some other plants in Hawaii. JOHNSON (**6**) confirmed KELLEY's finding and found that chlorotic pineapples were restored to normality by spraying with an iron sulphate solution.

WALLACE and MANN (**8**) found considerable difference in the chemical composition of the ash of chlorotic and normal fruit trees. The percentage of ash in the dry matter, with one exception, was higher in the chlorotic than in the normal leaves.

The Chemistry Department of the Idaho Agricultural Experiment Station (**5**) associates the chlorosis of trees and shrubs with high calcium carbonate soils even though a considerable amount of iron is present. GILES (3) found that the chlorosis of pineapples in Porto Rico was the result of malnutrition, caused by an excessive amount of calcium carbonate in the soil. GILES and CARRERO (**4**) report that chlorotic rice plants may be restored to their normal color by spraying with ferrous sulphate.

BURGESS and POHLMAN (**2**), in studying the cause of citrus chlorosis, found that improper irrigation was a factor. Spraying the trees with an iron salt solution did not give beneficial results.

[1] Contribution from Montana State College, Agricultural Experiment Station, Paper no. 14, Journal series.

It is evident, from the literature cited, that numerous plants become chlorotic when grown in soils high in calcium carbonate, or manganese, or when improperly irrigated.

The first investigation of chlorosis at the Montana Station dates back to the spring of 1908, at which time the Departments of Chemistry and Botany treated a chlorotic cottonwood tree, growing on the College campus, with a 0.25 per cent. solution of ferrous sulphate. The tree was about ten inches in diameter and four liters of the iron solution were injected into the trunk. Improvement was rapid and the tree had fully recovered by the following spring. Since that time several cottonwood trees have been treated with a solution of ferrous sulphate with equally good results. A slight burning of the leaves occurred when a 0.5 per cent. solution was used and as much as four liters applied to trees eight to ten inches in diameter.

An investigation of apple trees was started in 1914 when the writer's attention was called to a condition affecting the orchards in certain sections of the Bitter Root Valley, the fruit section of the State. At the time of inspection the trees were making poor growth and the leaves were small and yellowish in color. It was found, on taking soil samples near the trees showing abnormal conditions, that the subsoil consisted quite largely of sand and gravel of various sizes cemented together by calcium carbonate. It was almost impossible to go through this formation with a pick or a crowbar. The average analyses of several samples gave the following results: First foot, nitrogen, 0.095 per cent., calcium 8.05 per cent., phosphorus 0.105 per cent., and potassium 0.29 per cent.; second foot, nitrogen 0.057 per cent., calcium 14.62 per cent., phosphorus 0.083 per cent., potassium 0.20 per cent., and iron 0.35 per cent.

The analyses indicate that the soil was fairly well supplied with nitrogen and phosphorus, with an abnormally large supply of calcium and a rather low amount of potassium. The percentage of iron was considerably less than is found in fertile soils of the state.

No further investigation was made until the fall of 1922, when our attention was again called to this trouble by the State Horticulturist, who pointed out that chlorosis in the Bitter Root Valley might prove as injurious to that fruit section as had the fruit-tree leaf-roller, which had just made its appearance and was doing considerable damage. It was found that the greatest injury was occurring in a high-lime soil area.

In the orchard selected for conducting the experiments many of the trees were not only failing to make normal growth, but several were dead. The leaves on the chlorotic trees were small and yellow, and the trees had made little or no growth. The poor condition of the trees had been attributed by some people to high water-table and a water-logged condition, while others thought it was the result of too much alkali in the soil. How-

ever, a hole dug five feet deep near a chlorotic tree failed to reach the water-table and an analysis of the soil revealed the presence of very little alkali salts, not enough to cause tree injury. An average analysis of the second foot of soil showed a calcium content of 14.53 per cent. and 1.0 per cent. of iron. The samples were not analyzed for nitrogen, phosphorus, and potassium, since we believed that they would not vary materially from the analyses given.

An inspection of this orchard, made a few days after it had been irrigated, showed an apparent water-table at a depth ranging from two to three feet. This condition, while only temporary, undoubtedly had the effect of decreasing the supply of available nitrogen, which would result in lightening the color of the leaves.

Treatment of trees

After making a study of the soil and trees it was decided that the chlorotic condition was probably caused by too much lime in the soil, and that treatment with iron should prove beneficial. The first treatments were made in August, 1922, when 500 cc. of a 0.25 per cent. solution of ferrous sulphate were injected into each of two trees with yellow leaves that had failed to make a normal growth. A hole, one-fourth inch in diameter, was bored into the sap wood of the trunk of the tree, into which an L-shaped gas pipe was fitted. One end of a rubber tube was slipped over the end of the gas pipe and the other end was attached to a wide-mouthed bottle containing iron sulphate solution. The bottle was inverted and hung to a branch of the tree about four feet above the gas pipe in the trunk. To admit air into the bottle a U-shaped glass tube was used, one end being pushed through a rubber stopper to the bottom of the bottle and the other end projecting to a little above the bottom on the outside. By this method the iron solution was injected into the tree. Two other trees were treated by digging a circular trench about one foot deep and four feet from the trunk. Five pounds of ferrous sulphate were scattered in the trench, after which it was covered with earth and watered. Another set of two trees was sprayed with a 0.5 per cent. solution of ferrous sulphate. Several other trees showing a decidedly chlorotic condition were treated with a complete fertilizer to determine if a lack of plant food might be the cause.

Results

An inspection made in the fall showed that neither the soil application nor the spray treatment had improved the chlorotic condition of the trees. The injection of ferrous sulphate had only slightly improved the color of the leaves formed at the time of treatment, but the leaves which had formed after treatment were of a green color and normal in appearance. Figure 1

gives a fair idea of the difference in the appearance of the leaves before and after treatment. The upper three leaves were formed after treatment.

FIG. 1. Leaves of apple, showing recovery from chlorosis. The upper three leaves produced after treatment.

An inspection made the following June showed no beneficial effect from the spray, soil or fertilizer treatment, while the two trees into which ferrous sulphate had been injected were normal in appearance and remained so.

From these results it was evident that a solution of ferrous sulphate injected into a tree would restore the leaves to their normal color. Another test was undertaken in May, 1924, when twelve trees with yellow leaves were treated with ferrous sulphate or ferric nitrate. In some cases the iron salts were injected into the trunk of the tree for the purpose of restoring the whole tree to normal condition, while in other instances, the injection was made into one branch of the tree so that a comparison could be made between the color of the leaves on the treated and the untreated branches. In addition to injecting an iron solution into the tree, a one-half inch hole, sloping downward, was bored into the tree and either ferrous sulphate or ferric nitrate salts inserted and water added until the salts were dissolved. Another yet simpler method of supplying the iron, one that any orchardist could use, was also tried, *viz.*, driving iron nails into the trees. Heavy finishing nails, about one to one and one-fourth inches long, were used for this purpose. Care was used in driving the nails into the tree to avoid injuring the bark, and a nail-set was used to drive the nails so that the heads would be completely under the bark. In the case of branches some eight or ten nails were driven in. Where the nails were driven into the trunk as many as fifteen or twenty were used. The nails did not give as rapid

TABLE I

CONDITION OF TREES, TREATMENT AND RESULTS

TREE NUMBER — CONDITION OF TREE	TREATMENT	RESULTS
1. All leaves were yellow, general condition bad	Worst branch treated with 2 gm. $Fe(NO_3)_3$ crystals	Some improvement during summer. Following year the leaves were all yellow. Tree pulled
2. Leaves were yellow	2 gm. $FeSO_4$ crystals put into hole in trunk	Recovery was complete
3. Leaves were yellow	3 gm. $Fe(NO_3)_3$ crystals put into hole in trunk	Recovery was complete
4. Decidedly chlorotic	Worst branch treated with 3 gm. $Fe(NO_3)_3$ crystals	Branch recovered, remainder of tree remained chlorotic
5. Part of the leaves were yellow	30 cc. of 12.5 per cent. solution of $Fe(NO_3)_3$ injected into trunk	Recovery was complete
6. Decidedly chlorotic	5 gm. $FeSO_4$ crystals put into one branch. One year later, iron nails were driven into trunk	Treated branch recovered first. Last inspection found an almost complete recovery
7. Decidedly chlorotic	Worst branch treated with 3 gm. $FeSO_4$ crystals. One year later iron nails driven into trunk	Treated branch recovered first. Balance of the tree recovered slowly, but completely
8. Decidedly chlorotic	500 cc. 0.25 per cent. solution of $Fe(NO_3)_3$ injected into one branch. One year later iron nails driven into trunk	Slight improvement the first year. Last inspection found the tree still chlorotic
9. Worst physical condition of any of the trees treated	First, 5 lb. $FeSO_4$, second, 500 cc. 0.25 per cent. solution $FeSO_4$ injected into one branch. Third, complete fertilizer added to soil. Fourth, 5 gm. $FeSO_4$ crystals put into hole in trunk. Fifth, iron nails driven into trunk	Slow, but complete recovery
10. Decidedly chlorotic	500 cc. 0.25 per cent. solution of $FeSO_4$ injected into one branch. One year later, iron nails driven into trunk	Recovery was complete
11. All leaves were yellow	2 gm. $FeSO_4$ crystals put into trunk	Recovery was complete
12. All leaves were yellow	500 cc. 0.25 per cent. solution of $FeSO_4$ injected into one branch	Leaves on treated branch became normal, those on remaining branches remained chlorotic

action as when the iron was put into the tree in solution, yet the final results were as satisfactory. The accompanying table describes the condition of each tree at the time of treatment, May 20, 1924, the kind of treatment and the results obtained.

In addition to the trees described in the table, four trees were selected for treatment with iron nails only. On June 1, 1925, iron nails were driven into the trunks of the trees. The leaves on all of the trees were yellow at the time of treatment. A slight improvement was noted when inspected on August 18, 1926. On June 21, 1927, when last inspected, recovery was complete.

Summary

Cottonwood trees are susceptible to chlorosis, which can be overcome by treatment with iron salts.

Apple trees grown in high lime soils are subject to chlorosis. The lime evidently prevents the iron in the soil from becoming available. The fact that, in most cases, iron salts restored trees to normality indicates a lack of *available* iron in the soil.

Spraying with a 0.5 per cent. solution of ferrous sulphate, or applying ferrous sulphate to the soil, did not improve the chlorotic condition of the trees.

Treatment internally with iron salts, both as dilute solutions and as solid salts, gave beneficial results.

Iron nails driven into the trunks of four trees gave beneficial results. The recovery of chlorotic trees treated with iron nails was not so rapid as when treated with iron salts, although it seemed to be as effective. The ease of application should appeal to orchardists.

MONTANA AGRICULTURAL EXPERIMENT STATION,
BOZEMAN, MONTANA.

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SIERGEI PAVLOVITCH KOSTYTCHEV
1877–1931

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BRIEF PAPERS

SIERGEI PAVLOVITCH KOSTYTCHEV

(WITH ONE PLATE AND ONE FIGURE)

On August 31, 1931, there died in Crimea, Russia, the prominent plant physiologist, S. P. KOSTYTCHEV. His death came as a shock to his friends abroad, especially to those who attended in 1930 the Second International Soil Science Congress and who have learned to know personally this brilliant investigator.

KOSTYTCHEV was born in St. Petersburg in 1877, the son of a Professor, a prominent soil scientist. In 1896 he entered the local University, specializing in botany and especially in plant physiology, under D. I. IVANOVSKI. Upon graduation in 1900, he left for Western Europe, studying plant chemistry with E. SCHULZE in Zürich and with A. KOSSEL in Heidelberg. On his return to Russia in 1903, he became attracted by the brilliant investigations of V. I. PALLADIN, on plant respiration, and became the outstanding representative of PALLADIN'S school in Russia. His Master's thesis in 1907 dealt with "Investigations on the anaerobic respiration of plants," and his Doctor's thesis in 1910 on "Physiologo-chemical investigations of plant respiration." He was at first assistant in the Academy of Medicine in St. Petersburg; in 1910 he was appointed professor at the Technological Institute and in 1914 professor of plant physiology at the University. In 1923 he was elected a member of the Russian Academy of Sciences.

He is best known for his investigations on the rôle of anaerobic processes in normal respiration. During the germination of peas, in the absence of oxygen, alcohol was accumulated, while the products of incomplete fermentation of sugar by yeasts added to wheat seedlings were completely oxidized to CO_2. His later work was centered primarily upon the mechanism of alcoholic fermentation, especially the intermediary fermentation products during the last stages of the process, and the rôle of acetaldehyde in this process. His investigations of carbon assimilation by different types of plants as influenced by environmental conditions, of the rôle of zymase in alcoholic fermentation, of the relation of fermentation to respiration in yeasts and filamentous fungi, of the mechanism of nitrogen assimilation by fungi, and of the formation of ammonia in the process of nitrogen fixation by *Azotobacter,* have contributed materially to the advance of our knowledge of the physiology of higher plants and lower organisms. More recently, KOSTYTCHEV was placed in charge of the Division of Agricultural Microbiology at the Institute of Experimental Agronomy and later was made the chief of a new Institute of Microbiology of the Lenin Agricultural

FIG. 1. A group of investigators interested in the rôle of micro-organisms in soil processes and plant growth at the meeting of the Third Commission of the International Congress of Soil Science at Stockholm in 1929.

Academy, where he surrounded himself with a considerable number of young investigators, working on numerous problems in the field of agricultural microbiology.

He is best known in this country for his books on "Plant respiration" and "Plant physiology." He took an active part in organizing the program and the meetings of the Third Commission on Soil Microbiology in 1929, in Stockholm, and of the Second International Soil Science Congress in 1930, in Leningrad and Moscow. As Vice-President of this commission, he contributed greatly to making the meetings successful. His knowledge of foreign languages, his charming personality, his deep interest in his work, made him beloved and respected by all who came in contact with him.—SELMAN A. WAKSMAN, *New Jersey Agr. Exp. Sta., New Brunswick, N. J.*

SOME PHOTOPERIODIC AND TEMPERATURE RESPONSES OF THE RADISH

(WITH ONE FIGURE)

In connection with their studies on photoperiodism, GARNER and ALLARD (**2**) pointed out that the formation of tubers commonly results from excessively short days. The present brief experiment was performed to detect what influences, if any, temperature as well as length of day exerts upon the development of the storage organ of the radish.

Seeds of the radish, *Raphanus sativus*, of the Earliest Carmine Turnip variety, were planted in four pots on June 29, 1931. They were placed under the four following conditions: 25° C. and long day, 25° C. and short day, 15.5° C. and long day, 15.5° C. and short day. The temperatures were maintained in controlled glass chambers. The long day was the natural day length, about 15 hours. The short day was 7.5 hours (9 A. M. to 4:30 P. M.), the pots being covered with cardboard boxes the rest of the time. Since the temperature within the boxes did not rise more than 1.5° above the temperature without, the difference was considered negligible.

The percentage of thickened hypocotyls at low temperature was approximately the same under the two light conditions after about five weeks, but those with the shorter day length were more uniform and slightly smaller.

At the higher temperature the results were different: there were fewer thickened hypocotyls under short-day conditions than under long-day conditions. It was striking that while thickening was good with both light periods at low temperature, it was very poor with both light periods at high temperature. In the latter case the hypocotyl tended to assume a procumbent position; also the foliage was larger than at low temperature. It is pointed out by GARNER and ALLARD (**3**) that short-day illumination checks vegetative growth and causes an increase in tuber formation. From the present results it would seem that temperature must also be taken into account.

Microchemical tests (**1**) at the end of the day (4:30 P. M.) showed that all the thickened hypocotyls contained a little fructose and a great abundance of glucose (Fehling's solution). This is in accordance with NIGHTINGALE's report (**4**) that the radish stores sugars, not starch, in the storage organ. The small thickened hypocotyls, however, grown at high temperature and with a long day, showed an abundance of very small starch grains in the parenchymatous tissues (iodine potassium iodide). RIOLLE (**5**) reports that some varieties of radishes, especially Chinese ones, may contain a considerable amount of starch; but she also reports that very few French and Japanese varieties ever contain any starch, and then only very little. So it would appear here that a variety which does not form starch under

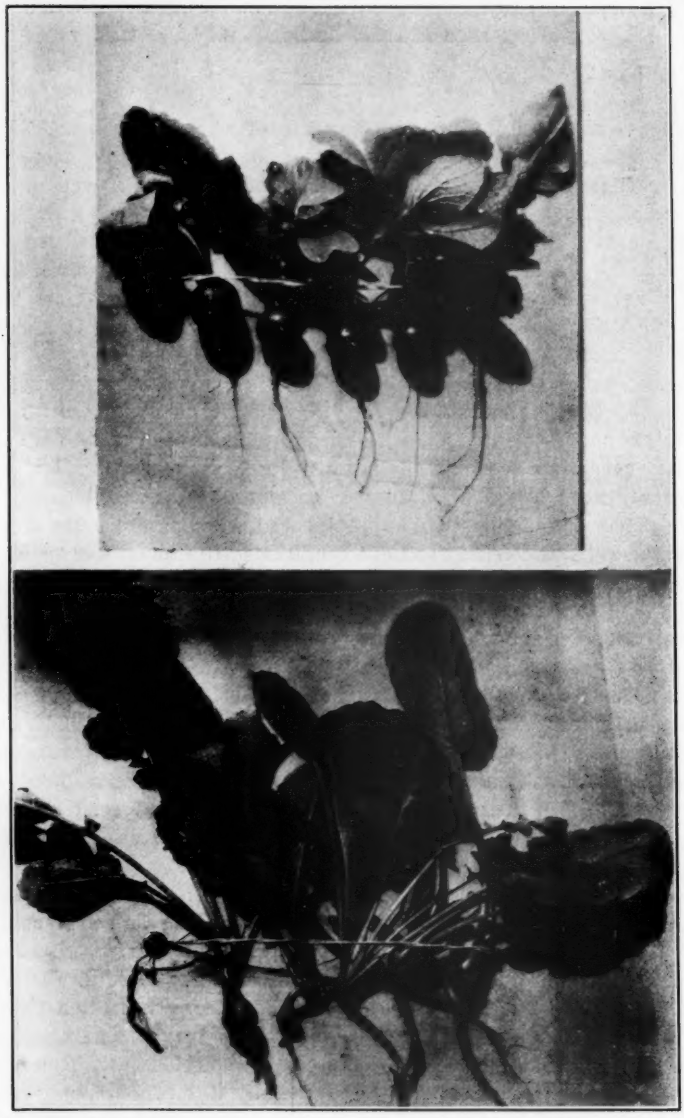

FIG. 1. Radishes grown under short-day conditions: above, low temperature; below, high temperature.

ordinary circumstances does do so under certain extreme conditions. May not this unexpected and unusual condition indicate that temperature, at least beyond the range normal for the radish, influences the form in which the carbohydrates are stored?

This experiment was kindly suggested by Dr. H. E. HAYWARD and guided with constant interest by Dr. C. A. SHULL.—THORA M. PLITT, *University of Chicago.*

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TOXICITY OF ROOT EXCRETIONS

(WITH ONE FIGURE)

From ancient times the belief has persisted that certain species of plants may give off through their roots excretions which are toxic to the roots of other plants. Although the evidence for this idea has never been very strong, it has been kept alive in the literature by occasional observations and investigation of alleged cases of the phenomenon. PICKERING'S[1] studies of the influence of grass roots upon the behavior of the apple are well known. The discovery of dihydroxystearic acid in wheat-sick soils was at one time interpreted as a case of toxic excretion. More recently FLETCHER[2] reported the extreme sensitivity of *Sesamum indicum* to the root excretions of *Andropogon sorghum* (*Sorghum vulgare*), and SEWELL[3]

[1] PICKERING, S. U. The effect of grass on apple trees. Jour. Roy. Agr. Soc. England **64**: 365–376. 1903.

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[2] FLETCHER, F. Toxic excreta of plants. Jour. Agr. Sci. **4**: 245–247. 1912.

[3] SEWELL, M. C. Effect of *Andropogon sorghum* on succeeding crops of *Triticum sativum vulgare*. Bot. Gaz. **75**: 1–26. 1923.

found that the roots of *A. sorghum* injured succeeding crops of wheat. He was able to obtain an extract from the soil in which the *Sorghum* was growing which was toxic to the wheat plant if the extract were prevented from becoming oxidized.

In some of these cases the alleged toxicity has been reinterpreted in terms of competition of root systems for nutrients such as nitrogen in the soil; in others, the toxicity can be interpreted in terms of the products of root decay in the soil. The case of *Sesamum indicum* mentioned by FLETCHER seemed very extraordinary. His photographs indicate a profound effect upon the growth of *Sesamum,* and he concludes that *Sesamum* cannot grow to maturity within 20 inches of *A. sorghum.* It is practically certain that not all of the influential factors were under control in FLETCHER'S field tests, if indeed any of them were. But the stunted growth was so remarkable that the writer had long desired to see the results of the *Sorghum* root excretions upon the *Sesamum* plant. The opportunity to make such studies was not presented until very recently. Through the courtesy of Prof. G. S. KULKARNI, and Prof. V. G. GOKHALE, of the Agricultural College, Poona, India, seeds of five varieties of the Indian jowar (*S. vulgare*) were obtained (Bedari, Fulgar, Gidgap, Maldandi, and Perio), as well as seeds of both the white- and black-seeded varieties of *Sesamum indicum.*

These seeds were first grown in pot cultures in the greenhouses of the Oregon State College[4] during the spring of 1931. Each of the varieties of *Sorghum,* and the two varieties of *Sesamum,* were grown alone as controls. There were five experimental cultures in which each variety of *Sorghum* was grown along with black sesame, and five in which white sesame accompanied the sorghums.

The soil used was a light sandy mixture which proved to be too light for very vigorous growth, but in every case the variety of *Sorghum* grew in association with the *Sesamum* plants without causing any apparent injury to the latter. At the end of about nine weeks the pots were examined for root growth. It was found that the roots of the *Sorghum* and *Sesamum* plants were growing together most intimately, without the slightest sign of abnormality of root development in the *Sesamum.* There was nothing to suggest any toxic action on the part of the *A. sorghum* roots.

After returning to the University of Chicago, a bed of *Sorghum* and *Sesamum* was planted about July 1, 1931, in one of the ground-level beds of the Botany greenhouses. Five rows of sorghum, one of each variety, were planted about 16 inches apart, running north and south. Between each two rows of *Sorghum* was planted a row of *Sesamum indicum,* white

[4] I am indebted to Professor H. P. BARSS and Dr. W. M. ATWOOD, and to the Administrative Officers of the Oregon State College, for many courtesies extended to me in connection with this and other problems.

or black, two rows of each being used. These were planted thus about 8 inches from the sorghums. The seeds germinated very promptly and the plants grew luxuriantly in the closest association. Figure 1 shows a photo-

FIG. 1. Three Indian varieties of *A. sorghum*, Gidgap, Fulgar, and Bedari, with *S. indicum*, black-seeded, growing between them.

graph of the plants when about half grown. There were no indications of toxicity of the roots of *A. sorghum* (*S. vulgare*) for the roots of *Sesamum indicum*. The plants continued to grow until they reached the blooming stage together, during the first week in October. Plants of both species were blossoming normally within 8 inches of each other. The sorghums at their best were over 3 meters tall, and the sesame plants more than a meter tall. It was necessary to thin the sorghums to prevent shading of the sesame plants, and to remedy lodging of the sorghums from low light intensity which was aggravated by the thick planting.

The results failed entirely to confirm the alleged toxicity of the sorghums for *S. indicum*. Whatever the behavior of these plants may be under the usual cultural conditions, and the edaphic and climatic conditions of India, in these greenhouse experiments there was no evidence of the toxicity of root excretions of *A. sorghum*. The results obtained tend to make one skeptical as to the reality of toxic root excretions emanating from the root systems of living plants. The burden of proof should surely rest with those who make the claim that such toxic excretions exist.—CHARLES A. SHULL, *University of Chicago.*

NOTES

New Orleans Meeting.—The eighth annual meeting of the American Society of Plant Physiologists was held at New Orleans from December 29, 1931, to January 1, 1932. It was well attended, and was one of the most satisfactory meetings in the history of the organization. Joint meetings were held with the horticulturists, and with Section G of the A. A. A. S. The program committee had arranged the papers with some reference to the subject-matter, and large attendance was the rule. About sixty members and friends of the Society were present at the banquet. The occasion was one of delightful fellowship, and maintained the tradition that the plant physiologists have one of the most attractive social events of the meetings.

Many matters of business were considered, and some important decisions were made, which will concern the future welfare of the Society. A few of the most important matters are reported briefly in this number of PLANT PHYSIOLOGY.

Incorporation.—The Committee on Incorporation, Dr. WALTER THOMAS, *chairman;* Dr. BURTON E. LIVINGSTON, and Dr. J. B. OVERTON, reported the results of the investigation of the advantages and disadvantages of incorporation of scientific societies. Weighing the matter carefully, the committee recommended incorporation either under the laws of the state of Wisconsin, or under those of the District of Columbia. After discussion, it was voted by the Society to incorporate, preferably under the laws of the District of Columbia. This change in legal status will prove advantageous in several ways. It will no doubt make it simpler to accept and administer bequests. It will also relieve the individual members of the Society from financial responsibility for the corporation's debts, if it should have any. Moreover, it will be conducive to stability and permanence of organization. The officers of the Society, authorized to take this step, will proceed at once with the legal steps necessary to incorporation.

Editorial Assistance.—The Society voted funds for an assistant to the editor of PLANT PHYSIOLOGY. This action is very much appreciated in the editorial office. The journal has almost doubled in size since its initial volume, and the assistance provided will lighten the burden very materially. A very capable assistant has been secured, with arrangements which we hope may be permanently satisfactory. With this help it should be possible in the immediate future to begin issuing the journal during the month for which is it dated.

Secretarial Office.—Expenses of the Secretary's office tend to exceed the amount allowed under the constitution. As the Society has accumulated reserve funds which are unassigned to other purposes, it was voted to allow a limited use of interest from such unassigned funds to supplement the current income from membership fees. The largest drain on the Secretary's funds is the International Address list, which of course should be issued at about five-year intervals. Publication of the membership list might be made a biennial matter instead of annual, without very serious loss in efficiency.

Life Membership Award.—The seventh award of the Charles Reid Barnes life membership in the Society was announced at the annual dinner on January 1. The announcement was made by Dr. R. H. CARR, chairman of the committee, and the member honored is Dr. CHARLES ORVILLE APPLEMAN, of the University of Maryland.

Dr. APPLEMAN was born at Millville, Pa., December 6, 1878. He holds degrees from the Bloomsburg (Pa.) State Normal School, Dickinson College, and The University of Chicago (Ph.D., 1910). He was Professor of Biology at Lombard College, 1904–1908, and in 1910 was appointed plant physiologist in the Maryland Agricultural Experiment Station. Since 1917 he has been Professor of Plant Physiology and Biochemistry in the University of Maryland, and Dean of the Graduate School. He now heads the Department of Botany in the institution he has served so faithfully.

An incident brought out in Dr. APPLEMAN's response to the award is the fact that he was a member of the anxious group of students who waited in vain for a lecture at the time of Dr. BARNES's fatal accident. It is very fitting that the most distinguished member of that group has been added to the roll of life members who form a living memorial to BARNES, who is remembered by all who knew him, as a great teacher, a cordial friend, and a keen critic in the field of plant physiology.

Stephen Hales Prize.—The third Stephen Hales Prize is to be awarded at the ninth annual meeting of the Society at Atlantic City, New Jersey, in 1932. The committee in charge of the selection consists of the following members: Dr. A. L. BAKKE, *chairman;* Dr. D. R. HOAGLAND, and Dr. W. W. GARNER. The award is made every second year to some investigator in North America who has done work considered by the committee to be worthy of this honor. Announcement of the award is made at the annual dinner.

Summer Meeting.—A program of unusual interest is being arranged for the summer meeting of the American Society of Plant Physiologists,

which is to be held at the University of Wisconsin, Madison, on July 11, 12, and 13, 1932. This meeting will occur jointly with that of the Corn Belt Section of the American Society of Agronomy. The program will be built around the environmental relations of plants from the view-point of biochemists, physiologists, agronomists, pathologists, and bacteriologists. It is proposed to confine the discussion of the first few sessions to a number of subjects of basic interest which will permit the utilization of the more significant examples of field, greenhouse, and laboratory work as illustrative material for presenting principles and technique. On the last day a field trip is being arranged to visit the outlying experimental areas in Wisconsin which represent a wide range of environmental conditions. The local representative of the Society for this meeting at the University of Wisconsin, is Dr. L. F. GRABER, of the Agronomy Department at Wisconsin.

Accommodations at Madison.—Members attending the summer meeting in July may be able to secure accommodations at the University of Wisconsin dormitories at $1.50 per day, with meals at 20 to 60 cents. The principal hotels and their rates are as follows:

	Single	Double
Loraine Hotel, 123 West Washington Ave.,	$2.00–$3.00 up,	$3.00–$5.00
New Park Hotel, 22 S. Carroll St.	1.50– 2.00	2.50– 3.00
Belmont Hotel, 31 N. Pinckney	2.00– 2.50	3.50– 4.00
Cardinal Hotel, 416 E. Wilson	1.50– 2.00	2.00– 2.50

Summer Meeting, A. A. A. S.—The summer meeting of the American Association for the Advancement of Science is to be held at Syracuse during the week beginning June 20th, 1932. These summer meetings provide most pleasant features, and the Finger Lake region is enticingly beautiful. The American Society of Plant Physiologists has made no plans to participate in this meeting, since the meeting at Madison had been arranged before the Syracuse meeting was announced. Members of the Society in the surrounding territory may find it possible to participate in a local way. Notices as to fares, accommodations, etc., may be obtained from *Science*.

Portraits.—We are greatly indebted to Dr. G. J. PEIRCE, of Stanford University, for the privilege of using the portrait of Sachs published in the January number of PLANT PHYSIOLOGY. The photographs of the Laboratory at Würzburg, one of which, with SACHS seated in his work room is published in this issue, have been secured through the efforts of Dr. F. M. ANDREWS, chairman of the Memorial Committee, and Dr. R. B. HARVEY. The frontispiece in this issue was sent to Prof. Andrews by Dr. BURGEFF, of the Botanical Institute, Würzburg.

The portraits which have been published in PLANT PHYSIOLOGY will be furnished while they last, at 10 cents each, with 15 cents additional for postage, packing and exchange. This is at the rate of $1.45 for 13, $1.55 for 14, etc. Broken lots can be ordered at the same rate provided the charge for postage, etc., is included.

Chemical Methods.—There are still a few sets of the pamphlets published by the Committee on Chemical Methods available to plant physiologists who would like to own a set for personal use. They can be obtained from Dr. W. E. TOTTINGHAM, President of the Society, at the University of Wisconsin.

Program Committee.—The Program Committee for the Atlantic City meeting has been appointed by President TOTTINGHAM, and consists of the following members: Dr. JOHN W. SHIVE, New Jersey Agricultural Experiment Station, *chairman;* Dr. GEORGE H. DUNGAN, University of Illinois; Dr. MARY E. REID, Washington, D. C.; Dr. C. O. APPLEMAN, University of Maryland; and Dr. RAYMOND KIENHOLZ, Connecticut Agricultural Experiment Station. The committee members will appreciate the cooperation of all other members in arranging for this meeting. Special SACHS programs are being planned as a part of the celebration of his centennial. The Memorial Committee is represented in the Program Committee in order to facilitate cooperation between the committees.

Agricultural Biochemistry.—A new work in this field has been published by John Wiley and Sons, New York. The authors are Professor R. A. DUTCHER, and Professor D. E. HALEY, of the Department of Agricultural and Biological Chemistry, Pennsylvania State College.

The material is presented in three parts. Part I is general and introductory, giving a history of the rise of agricultural chemistry, the chemistry of protoplasm, the properties and classification of the carbohydrates, lipides, and proteins. There is also a chapter on the physical state of matter, which includes the properties of solutions, nature of membranes, H-ion concentration, buffers, colloids, adsorption, etc., and a chapter on enzymes.

Part II considers the plant, germination, mineral nutrition, fertilizers and other soil factors, insecticides, and the formation and utilization of organic compounds. Part III deals with the animal foods, digestion, metabolism of tissues, protein quality, vitamins, etc.

The book is simple enough to serve as an introduction, and, in addition to covering the field, contains a summary of the valuable work done at the Pennsylvania State College on plant and animal nutrition. These dis-

tinctive features will be much appreciated by users of the work. It will be welcomed as a text suited to the student beginning his interest in biochemistry. The book is priced at $4.50 by the publishers.

Principles of Soil Microbiology.—The second edition of this splendid work by Dr. S. A. WAKSMAN, of the New Jersey Agricultural Experiment Station, has been published by the Williams and Wilkins Co., Baltimore, Maryland. It has been carefully revised, some chapters rewritten, and other chapters added which tie the work more closely to the field of plant nutrition. Some condensation and omission of material has permitted a book of about the same size as the original edition in 1927. The work has gained in value with the revision, and is the outstanding contribution in the field of soil microbiology. It is an indispensable reference work for the bacteriologist, soil scientist, and plant physiologist. The price is $10.00, the same as the first edition.